Renewable and Sustainable Energy Reviews 14 (2010) 557–577

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products

Liam Brennan [a,*], Philip Owende [a,b]

[a] Charles Parsons Energy Research Programme, Bioresources Research Centre, School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland
[b] School of Informatics and Engineering, Institute of Technology Blanchardstown, Blanchardstown Road North, Dublin 15, Ireland

ARTICLE INFO

Article history:
Received 21 August 2009
Accepted 9 October 2009

Keywords:
Microalgae
Biomass recovery
Bioenergy
Conversion
Photobioreactor
CO_2 sequestration

ABSTRACT

Sustainability is a key principle in natural resource management, and it involves operational efficiency, minimisation of environmental impact and socio-economic considerations; all of which are interdependent. It has become increasingly obvious that continued reliance on fossil fuel energy resources is unsustainable, owing to both depleting world reserves and the green house gas emissions associated with their use. Therefore, there are vigorous research initiatives aimed at developing alternative renewable and potentially carbon neutral solid, liquid and gaseous biofuels as alternative energy resources. However, alternate energy resources akin to first generation biofuels derived from terrestrial crops such as sugarcane, sugar beet, maize and rapeseed place an enormous strain on world food markets, contribute to water shortages and precipitate the destruction of the world's forests. Second generation biofuels derived from lignocellulosic agriculture and forest residues and from non-food crop feedstocks address some of the above problems; however there is concern over competing land use or required land use changes. Therefore, based on current knowledge and technology projections, third generation biofuels specifically derived from microalgae are considered to be a technically viable alternative energy resource that is devoid of the major drawbacks associated with first and second generation biofuels. Microalgae are photosynthetic microorganisms with simple growing requirements (light, sugars, CO_2, N, P, and K) that can produce lipids, proteins and carbohydrates in large amounts over short periods of time. These products can be processed into both biofuels and valuable co-products.

This study reviewed the technologies underpinning microalgae-to-biofuels systems, focusing on the biomass production, harvesting, conversion technologies, and the extraction of useful co-products. It also reviewed the synergistic coupling of microalgae propagation with carbon sequestration and wastewater treatment potential for mitigation of environmental impacts associated with energy conversion and utilisation. It was found that, whereas there are outstanding issues related to photosynthetic efficiencies and biomass output, microalgae-derived biofuels could progressively substitute a significant proportion of the fossil fuels required to meet the growing energy demand.

Contents

* Corresponding author. Tel.: +353 1 716 7458; fax: +353 1 716 7415.
E-mail address: liam.brennan@ucd.ie (L. Brennan).

1364-0321/$ – see front matter
doi:10.1016/j.rser.2009.10.009

1. Introduction

1.1. Energy outlook and salient environmental issues

In 2008 the annual world primary energy consumption was estimated at 11,295 million tonnes of oil equivalent (mtoe) [1]. Fossil fuels accounted for 88% of the primary energy consumption, with oil (35% share), coal (29%) and natural gas (24%) as the major fuels, while nuclear energy and hydroelectricity account for 5% and 6% of the total primary energy consumption, respectively [1]. Given the current technological progress, potential reserves, and increased exploitation of newer unconventional reserves (e.g. for natural gas), it is highly probable that fossil fuels will continue to be available at low cost for a considerable period of time; albeit with the variations in the security of supply arising from geo-political developments, from time to time [2,3]. Unfortunately, the potential threat of global climate change has increased, and for a major part, this has been attributed to greenhouse gas emissions from fossil fuel usage [4]. The associated climatic change projections could have major consequences for nature as well as human systems [5], which creates uncertainty regarding the sustainability of current fossil fuel use, not only in relation to the finiteness of the resource, but also on the negative effects of CO_2 emissions.

Fossil fuels are the largest contributor of greenhouse gases (GHGs) to the biosphere, and in 2006 associated CO_2 emissions were 29 Gtonnes [6]. It is estimated that natural processes remove only about 12 Gtonnes, therefore, compatible mitigation strategies are required to neutralise the excess CO_2 [7]. With the increase in anthropogenic GHG emissions, mainly due to large scale use of fossil fuels for transport, electricity and thermal energy generation, it has become increasingly important to develop abatement techniques and adopt policies to minimise impacts of global warming. The Kyoto Protocol of 1997 called for a 5.2% reduction in GHG emissions worldwide from 1990 values [8]. To meet the agreed target, a selection of a range of effective technologies, including chemical and biological CO_2 mitigation possibilities, has been a focus of research.

The overall implication is therefore a need for enhancement of global strategies for energy security and mitigation of CO_2-energy related emissions, for which the salient strategies include, *inter alia*, the need for: increased energy efficiency (i.e. decreasing energy use per unit of product, process or service); increased use of clean fossil energy (i.e. use of fossil fuels coupled with CO_2 separation from flue gases and injection into underground reservoir for gradual release), and; increased use of renewable energy (i.e. development of CO_2-neutral energy resources). Given the necessary CO_2 emission targets, and the potential of each of the outlined strategies to the timely reduction of CO_2 emissions to 'safe levels', it has been argued that the three outlined strategies will have to be employed in order to tackle the progression of climatic change [9].

1.2. Development of biofuel resources

In recent years, the use of liquid biofuels in the transport sector has shown rapid global growth, driven mostly by policies focused on achievement of energy security, and mitigation of GHG emissions [10]. First generation biofuels which have now attained economic levels of production, have been mainly extracted from food and oil crops including rapeseed oil, sugarcane, sugar beet, and maize [11] as well as vegetable oils and animal fats using conventional technology [12]. It is projected that the growth in production and consumption of liquid biofuels will continue [2], but their impacts towards meeting the overall energy demands in the transport sector will remain limited due to: competition with food and fibre production for the use of arable land, regionally constrained market structures, lack of well managed agricultural practices in emerging economies, high water and fertiliser requirements, and a need for conservation of bio-diversity [13].

Typically, the use of first generation biofuels has generated a lot of controversy, mainly due to their impact on global food markets and on food security, especially with regards to the most vulnerable regions of the world economy. This has raised pertinent questions on their potential to replace fossil fuels and sustainability of their production [14]. For example, apart from the risk that higher food prices may have severe negative implications on food security, the demand for biofuels could place substantial additional pressure on the natural resource base, with potentially harmful environmental and social consequences. Currently, about 1% (14 million hectares) of the world's available arable land is used for the production of biofuels, providing 1% of global transport fuels. Clearly, increasing that share to anywhere near 100% is impractical owing to the severe impact on the world's food supply and the large areas of production land required [15]. The advent of second generation biofuels is intended to produce fuels from the whole plant matter of dedicated energy crops or agricultural residues, forest harvesting residues or wood processing waste [14], rather than from food crops. However, the technology for conversion in the most part has not reached the scales for commercial exploitation which has so far inhibited any significant exploitation [11].

Conditions for a technically and economically viable biofuel resource are that [16]: it should be competitive or cost less than petroleum fuels; should require low to no additional land use; should enable air quality improvement (e.g. CO_2 sequesteration), and; should require minimal water use. Judicious exploitation of microalgae could meet these conditions and therefore make a significant contribution to meeting the primary energy demand, while simultaneously providing environmental benefits [8].

1.3. Potential role of biofuels from microalgae

In this review, the definition of microalgae covers all unicellular and simple multi-cellular microorganisms, including both prokaryotic microalgae, i.e. cyanobacteria (*Chloroxybacteria*), and eukaryotic microalgae, e.g. green algae (*Chlorophyta*), red algae (*Rhodophyta*) and diatoms (*Bacillariophyta*). The advantages of using microalgae-derived biofuels are: (1) microalgae are capable of all year round production, therefore, oil productivity of microalgae cultures exceeds the yield of the best oilseed crops, e.g. biodiesel yield of 12,000 l ha^{-1} for microalgae (open pond production) compared with 1190 l ha^{-1} for rapeseed [17]; (2) they grow in aqueous media, but need less water than terrestrial crops therefore reducing the load on freshwater sources [18]; (3) microalgae can be cultivated in brackish water on non-arable land, and therefore may not incur land-use change, minimising associated environmental impacts [19], while not compromising the production of food, fodder and other products derived from crops [20]; (4) microalgae have a rapid growth potential and many species have oil content in the range of 20–50% dry weight of biomass, the exponential growth rates can double their biomass in periods as short as 3.5 h [20–22]; (5) with respect to air quality maintenance and improvement, microalgae biomass production can effect biofixation of waste CO_2 (1 kg of dry algal biomass utilise about 1.83 kg of CO_2) [20]; (6) nutrients for microalgae cultivation (especially nitrogen and phosphorus) can be obtained from wastewater, therefore, apart from providing growth medium, there is dual potential for treatment of organic effluent from the agri-food industry [23]; (7) algae cultivation does not require herbicides or pesticides application [24]; (8) they can also produce valuable co-products such as proteins and residual biomass after oil extraction, which may be used as feed or fertilizer [22], or fermented to produce ethanol or methane [25]; (9) the biochemical composition of the algal biomass can be modulated by varying growth conditions, therefore, the oil yield may be significantly enhanced [26], and; (10) microalgae are capable of photobiological production of 'biohydrogen' [27]. The outlined combination of potential biofuel production, CO_2 fixation, biohydrogen production, and bio-treatment of wastewater underscore the potential applications of microalgae.

Despite its inherent potential as a biofuel resource, many challenges have impeded the development of algal biofuel technology to commercial viability that could allow for sustainable production and utilisation. They include: (1) species selection must balance requirements for biofuel production and extraction of valuable co-products [28]; (2) attaining higher photosynthetic efficiencies through the continued development of production systems [29]; (3) development of techniques for single species cultivation, evaporation reduction, and CO_2 diffusion losses [30]; (4) potential for negative energy balance after accounting for requirements in water pumping, CO_2 transfer, harvesting and extraction [31]; (5) few commercial plants in operation, therefore, there is a lack of data for large scale plants [32]; (6) incorporating flue gases which are unsuitable in high concentration owing to the presence of poisonous compounds such as NO_x and SO_x [33].

Sustainability is key to natural resource management or exploitation and it involves operational, environmental and socio-economic considerations; all of which are interdependent. This review outlines the state-of-the-art in biofuel production from microalgae. The uniqueness of the review is in its coverage of the integrated process chain and its interdependencies from algal biomass production, biofuel and co-products recovery processes, and algae-based CO_2 mitigation and wastewater treatment. It identifies the knowledge gaps within each area which can be targeted for focused research and innovation aimed at sustainable development of algae-based biofuel technologies.

2. Biology of microalgae

Algae are recognised as one of the oldest life-forms [34]. They are primitive plants (thallophytes), i.e. lacking roots, stems and leaves, have no sterile covering of cells around the reproductive cells and have chlorophyll *a* as their primary photosynthetic pigment [35]. Algae structures are primarily for energy conversion without any development beyond cells, and their simple development allows them to adapt to prevailing environmental conditions and prosper in the long term [34].

Prokaryotic cells (cyanobacteria) lack membrane-bound organelles (plastids, mitochondria, nuclei, Golgi bodies, and flagella) and are more akin to bacteria rather than algae. Eukaryotic cells, which comprise of many different types of common algae, do have these organelles that control the functions of the cell, allowing it to survive and reproduce. Eukaryotes are categorised into a variety of classes mainly defined by their pigmentation, life cycle and basic

cellular structure [36]. The most important classes are: green algae (*Chlorophyta*), red algae (*Rhodophyta*) and diatoms (*Bacillariophyta*). Algae can either be autotrophic or heterotrophic; the former require only inorganic compounds such as CO_2, salts and a light energy source for growth; while the latter are non-photosynthetic therefore require an external source of organic compounds as well as nutrients as an energy source. Some photosynthetic algae are mixotrophic, i.e. they have the ability to both perform photosynthesis and acquire exogenous organic nutrients [35]. For autotrophic algae, photosynthesis is a key component of their survival, whereby they convert solar radiation and CO_2 absorbed by chloroplasts into adenosine triphosphate (ATP) and O_2 the usable energy currency at cellular level, which is then used in respiration to produce energy to support growth [34,37].

3. Technologies for microalgal biomass production

Under natural growth conditions phototrophic algae absorb sunlight, and assimilate carbon dioxide from the air and nutrients from the aquatic habitats. Therefore, as far as possible, artificial production should attempt to replicate and enhance the optimum natural growth conditions.

The use of natural conditions for commercial algae production has the advantage of using sunlight as a free natural resource [38]. However, this may be limited by available sunlight due to diurnal cycles and the seasonal variations; thereby limiting the viability of commercial production to areas with high solar radiation. For outdoor algae production systems, light is generally the limiting factor [29]. To address the limitations in natural growth conditions with sunlight, artificial means employing fluorescent lamps are almost exclusively used for the cultivation of phototrophic algae at pilot scale stages [39]. Artificial lighting allows for continuous production, but at significantly higher energy input. Frequently the electricity supply for artificial lighting is derived from fossil fuels thus negating the primary aim of developing a price-competitive fuel and increasing the systems carbon footprint. For choosing an artificial light source, it important to understand the absorption spectra of major algal accessory pigments present in various quantities in different algal groups. For example, diatoms generally have photosynthetic pigments that include chlorophylls *a* and *c*, and fucoxanthin whereas green algae contain chlorophylls *a* and *b*, and zeaxanthin.

Microalgae can fix CO_2 from three different sources, namely: CO_2 from the atmosphere; CO_2 in discharge gases from heavy industry, and; CO_2 from soluble carbonates [8]. Under natural growth conditions, microalgae assimilate CO_2 from the air (contains 360 ppmv CO_2). Most microalgae can tolerate and utilise substantially higher levels of CO_2, typically up to 150,000 ppmv [7,40]. Therefore, in common production units, CO_2 is fed into the algae growth media either from external sources such as power plants [33,41–44] or in the form of soluble carbonates such as Na_2CO_3 and $NaHCO_3$ [45,46].

Other inorganic nutrients required for algae production include nitrogen, phosphorus and silicon [47]. While some algae species can fix nitrogen from the air in the form of NO_x [48,49], most microalgae require it in a soluble form with urea being the best source [50]. Phosphorus is of lesser importance and is required in very small amounts during algal growth cycle [51], but must be supplied in excess of basic requirement because phosphates ions bond with metals ions, therefore, not all the added P is bioavailable [20]. Importance of silicon is confined to productive growth of certain groups of algae such as diatoms [52].

This review considers three distinct algae production mechanisms, including photoautotrophic, heterotrophic and mixotrophic production, all of which follow the natural growth processes. Photoautotrophic production is autotrophic photosynthesis, heterotrophic production requires organic substances (e.g. glucose) to stimulate growth, while some algae strains can combine autotrophic photosynthesis and heterotrophic assimilation of organic compounds in a mixotrophic process.

3.1. Photoautotrophic production

Currently, photoautotrophic production is the only method which is technically and economically feasible for large-scale production of algae biomass for non-energy production [53]. Two systems that have been deployed are based on open pond and closed photobioreactor technologies [54]. The technical viability of each system is influenced by intrinsic properties of the selected algae strain used, as well as climatic conditions and the costs of land and water [55].

3.1.1. Open pond production systems

Algae cultivation in open pond production systems has been used since the 1950s [54]. These systems can be categorised into natural waters (lakes, lagoons, and ponds) and artificial ponds or containers. Raceway ponds are the most commonly used artificial system [56]. They are typically made of a closed loop, oval shaped recirculation channels (Fig. 1), generally between 0.2 and 0.5 m deep, with mixing and circulation required to stabilize algae growth and productivity. Raceway ponds are usually built in concrete, but compacted earth-lined ponds with white plastic have also been used. In a continuous production cycle, algae broth and nutrients are introduced in front of the paddlewheel and circulated through the loop to the harvest extraction point. The paddlewheel is in continuous operation to prevent sedimentation. The microalgae's CO_2 requirement is usually satisfied from the surface air, but submerged aerators may be installed to enhance CO_2 absorption [57].

Compared to closed photobioreactors (Table 1), open pond is the cheaper method of large-scale algal biomass production. Open pond production does not necessarily compete for land with existing agricultural crops, since they can be implemented in areas with marginal crop production potential [58]. They also have lower energy input requirement [24], and regular maintenance and cleaning are easier [30] and therefore may have the potential to return large net energy production [24]. In 2008, the unit cost of producing *Dunaliella salina*, one of the commonly cultivated algae strains, in an open pond system was about €2.55 per kilogram of dry biomass [59], which was considered to be too high to justify production for biofuels.

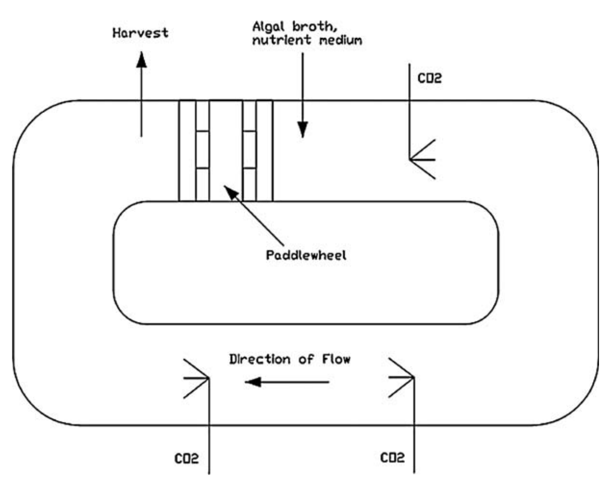


Fig. 1. Plan view of a raceway pond. Algae broth is introduced after the paddlewheel, and completes a cycle while being mechanically aerated with CO_2. It is harvested before the paddlewheel to start the cycle again (adapted from Chisti [20]).

Table 1
Advantages and limitations of open ponds and photobioreactors.

Production system	Advantages	Limitations
Raceway pond	Relatively cheap Easy to clean Utilises non-agricultural land Low energy inputs Easy maintenance	Poor biomass productivity Large area of land required Limited to a few strains of algae Poor mixing, light and CO_2 utilisation Cultures are easily contaminated
Tubular photobioreactor	Large illumination surface area Suitable for outdoor cultures Relatively cheap Good biomass productivities	Some degree of wall growth Fouling Requires large land space Gradients of pH, dissolved oxygen and CO_2 along the tubes
Flat plate photobioreactor	High biomass productivities Easy to sterilise Low oxygen build-up Readily tempered Good light path Large illumination surface area Suitable for outdoor cultures	Difficult scale-up Difficult temperature control Small degree of hydrodynamic stress Some degree of wall growth
Column photobioreactor	Compact High mass transfer Low energy consumption Good mixing with low shear stress Easy to sterilise Reduced photoinhibition and photo-oxidation	Small illumination area Expensive compared to open ponds Shear stress Sophisticated construction

Open pond systems, require highly selective environments due to inherent threat of contamination and pollution from other algae species and protozoa [29]. Monoculture cultivation is possible by maintenance of extreme culture environment, although only a small number of algae strains are suitable. For example, the species *Chlorella* (adaptable to nutrient-rich media), *D. salina* (adaptable to very high salinity) and *Spirulina* (adaptable to high alkalinity) thrive under such examples of extreme environments [54]. An example of large-scale monoculture cultivation is the production of *D. salina* for β-carotene in the extremely halophilic waters of Hutt-Lagoon, Western Australia [29]. However, long production periods for such approaches do not necessarily exclude bacterial and other biological contaminants [60].

In respect to biomass productivity, open pond systems are less efficient when compared with closed photobioreactors [20]. This can be attributed to several determining factors, including, evaporation losses, temperature fluctuation in the growth media, CO_2 deficiencies, inefficient mixing, and light limitation. Although evaporation losses make a net contribution to cooling, it may also result in significant changes to ionic composition of the growth medium with detrimental effects on algae growth [32]. Temperature fluctuations due to diurnal cycles and seasonal variations are difficult to control in open ponds [20]. Potential CO_2 deficiencies due to diffusion into the atmosphere may result in reduced biomass productivity due to less efficient utilisation of CO_2. Also, poor mixing by inefficient stirring mechanisms, may result in poor mass CO_2 transfer rates causing low biomass productivity [30]. Light limitation due to top layer thickness may also incur reduced biomass productivity. However, enhancing light supply is possible by reducing layer thickness; using thin layer inclined types of culture systems, and improved mixing can minimise impacts to enhance biomass productivity [20,30,32,61].

High algae biomass production rates are achievable with open pond systems. However, there are still inconsistencies in the production rates reported in literature (Table 2). Jiménez et al. [56] extrapolated an annual dry weight biomass production rate of 30 tonnes per hectare using data from a 450 m^2 and 0.30 m deep raceway pond system producing biomass dry weight of 8.2 g m^{-2} per day in Malaga, Spain. Using similar depth of culture, and biomass concentrations of up to 1 g l^{-1}, Becker [62] estimated dry biomass productivity in the range of 10–25 g m^{-2} per day. However, the only open pond system for large-scale production that has achieved such high biomass productivity is the inclined system developed by Setlik et al. [61] for the production of *Chlorella*. In this system, a biomass concentration of higher than 10 g l^{-1} was achieved, with extrapolated productivity of 25 g m^{-2} per day. Weissman and Tillett [63] operated an outdoor open pond (0.1 ha) in New Mexico, USA, and attained an average annual dry

Table 2
Biomass productivity figures for open pond production systems.

Algae species	X_{max} (g l^{-1})	P_{aerial} (g m^{-2} day^{-1})	P_{volume} (g l^{-1} day^{-1})	PE (%)	Reference
Chlorella sp.	10	25	–	–	[61]
N/A	0.14	35	0.117	–	[20]
Spirulina platensis	–	–	0.18	–	[214]
Spirulina platensis	0.47	14	0.05	–	[56,80]
Haematococcus pluvialis	0.202	15.1	–	–	[77]
Spirulina	1.24	69.16	–	–	[215]
Various	–	19	–	–	[63]
Spirulina platensis	0.9	12.2	0.15	–	[216]
Spirulina platensis	1.6	19.4	0.32	–	[216]
Anabaena sp.	0.23	23.5	0.24	>2	[49]
Chlorella sp.	40	23.5	–	6.48	[91]
Chlorella sp.	40	11.1	–	5.98	[91]
Chlorella sp.	40	32.2	–	5.42	[91]
Chlorella sp.	40	18.1	–	6.07	[91]

Table 3
Biomass productivity figures for closed photobioreactors.

Species	Reactor type	Volume (l)	X_{max} (g l^{-1})	P_{aerial} (g m^{-2} day^{-1})	P_{volume} (g l^{-1} day^{-1})	PE (%)	Reference
Porphyridium cruentum	Airlift tubular	200	3	–	1.5	–	[217]
Phaeodactylum tricornutum	Airlift tubular	200	–	20	1.2	–	[218]
Phaeodactylum tricornutum	Airlift tubular	200	–	32	1.9	2.3	[65]
Chlorella sorokiniana	Inclined tubular	6	1.5	–	1.47	–	[67]
Arthrospira platensis	Undular row tubular	11	6	47.7	2.7	–	[219]
Phaeodactylum tricornutum	Outdoor helical tubular	75	–	–	1.4	15	[96]
Haematococcus pluvialis	Parallel tubular (AGM)	25,000	–	13	0.05	–	[74]
Haematococcus pluvialis	Bubble column	55	1.4	–	0.06	–	[220]
Haematococcus pluvialis	Airlift tubular	55	7	–	0.41	–	[220]
Nannochloropsis sp.	Flat plate	440	–	–	0.27	–	[221]
Haematococcus pluvialis	Flat plate	25,000	–	10.2	–	–	[77]
Spirulina platensis	Tubular	5.5	–	–	0.42	8.1	[222]
Arthrospira	Tubular	146	2.37	25.4	1.15	4.7	[223]
Chlorella	Flat plate	400	–	22.8	3.8	5.6	[43]
Chlorella	Flat plate	400	–	19.4	3.2	6.9	[43]
Tetraselmis	Column	ca. 1,000	1.7	38.2	0.42	9.6	[224]
Chlorococcum	Parabola	70	1.5	14.9	0.09	–	[225]
Chlorococcum	Dome	130	1.5	11.0	0.1	–	[225]

weight biomass production rate of 37 tonnes per hectare with a mixed species culture (four species), highest yields were confined to the 7 warmest months of the year.

3.1.2. Closed photobioreactor systems

Microalgae production based on closed photobioreactor technology is designed to overcome some of the major problems associated with the described open pond production systems. For example, pollution and contamination risks with open pond systems, for the most part, preclude their use for the preparation of high-value products for use in the pharmaceutical and cosmetics industry [30]. Also, unlike open pond production, photobioreactors permit culture of single-species of microalgae for prolonged durations with lower risk of contamination [20]. Closed systems include the tubular, flat plate, and column photobioreactors. These systems are more appropriate for sensitive strains as the closed configuration makes the control of potential contamination easier. Owing to the higher cell mass productivities attained (Table 3) harvesting costs can also be significantly reduced. However, the costs of closed systems are substantially higher than open pond systems [64].

Photobioreactors consist of an array of straight glass or plastic tubes as shown in Fig. 2 [30]. The tubular array captures sunlight

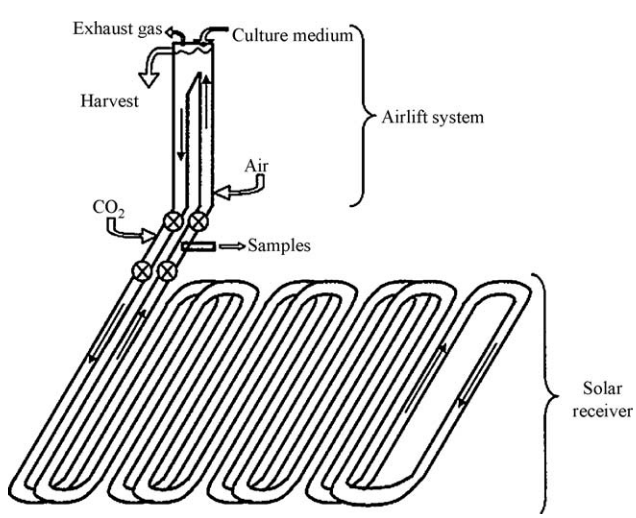


Fig. 2. Basic design of a horizontal tubular photobioreactor (adapted from Becker [62]). Two main sections: airlift system and solar receiver; the airlift systems allow for the transfer of O_2 out of the systems and transfer of CO_2 into the system as well as providing a means to harvest the biomass. The solar receiver provides a platform for the algae to grow by giving a high surface area to volume ratio.

and can be aligned horizontally [65], vertically [66], inclined [67] or as a helix [68], and the tubes are generally 0.1 m or less in diameter [20]. Algae cultures are re-circulated either with a mechanical pump or airlift system, the latter allowing CO_2 and O_2 to be exchanged between the liquid medium and aeration gas as well as providing a mechanism for mixing [69]. Agitation and mixing are very important to encourage gas exchange in the tubes.

Some of the earliest forms of closed systems are flat-plate photobioreactors [70] which have received much research attention due to the large surface area exposed to illumination [30] and high densities of photoautotrophic cells (>80 g l^{-1}) observed [71]. The reactors are made of transparent materials for maximum solar energy capture, and a thin layer of dense culture flows across the flat plate [71,72], which allows radiation absorbance in the first few millimetres thickness. Flat-plate photobioreactors are suitable for mass cultures of algae due to low accumulation of dissolved oxygen and the high photosynthetic efficiency achieved when compared to tubular versions [73].

Tubular photobioreactors have design limitations on length of the tubes, which is dependent on potential O_2 accumulation, CO_2 depletion, and pH variation in the systems [69]. Therefore, they cannot be scaled up indefinitely; hence, large-scale production plants are based on integration of multiple reactor units. However, tubular photobioreactors are deemed to be more suitable for outdoor mass cultures since they expose a larger surface area to sunlight. The largest closed photobioreactors are tubular, e.g. the 25 m^3 plant at Mera Pharmaceuticals, Hawaii [74], and the 700 m^3 plant in Klötze, Germany [32].

Column photobioreactors offer the most efficient mixing, the highest volumetric mass transfer rates and the best controllable growth conditions [69]. They are low-cost, compact and easy to operate. The vertical columns are aerated from the bottom, and illuminated through transparent walls [69], or internally [75]. Their performance compares favourably with tubular photobioreactors [76].

Closed photobioreactors have received major research attention in recent years. The noted proliferation of pilot-scale production using closed photobioreactors compared to open raceway ponds could be attributed to more rigorous process control and potentially higher biomass production rates, hence, potentially higher production of biofuel and co-product production.

3.1.3. Hybrid production systems

The hybrid two-stage cultivation is a method that combines distinct growth stages in photobioreactors and in open ponds. The first stage is in a photobioreactor where controllable conditions

Table 4
Biomass productivity figures for heterotrophic microalgae cultures.

Species	Product	Culture	X_{max} ($g\,l^{-1}$)	Total lipid (%)	P_{volume} ($g\,l^{-1}\,day^{-1}$)	Reference
Galdieria sulphuraria	C-phycocyanin	Continuous	83.3	–	50.0	[226]
Galdieria sulphuraria	C-phycocyanin	Fed-batch	109	–	17.50	[226]
Chlorella protothecoides	Biodiesel	Fed-batch	3.2	57.8	–	[227]
Chlorella protothecoides	Biodiesel	Fed-batch	16.8	55.2	–	[227]
Chlorella protothecoides	Biodiesel	Fed-batch	51.2	50.3	–	[227]
Chlorella	Docosahexaenoic acid	Fed-batch	116.2	–	1.02	[228]
Crypthecodinium cohnii	Docosahexaenoic acid	Fed-batch	109	56	–	[229]
Crypthecodinium cohnii	Docosahexaenoic acid	Fed-batch	83	42	–	[230]
Chlorella	N/A	Fed-batch	104.9	–	14.71	[228]
Chlorella protothecoides	Biodiesel	Fed-batch	15.5	46.1	–	[82]
Chlorella protothecoides	Biodiesel	Fed-batch	12.8	48.7	–	[82]
Chlorella protothecoides	Biodiesel	Fed-batch	14.2	44.3	–	[82]

minimise contamination from other organisms and favour continuous cell division. The second production stage is aimed at exposing the cells to nutrient stresses, which enhances synthesis of the desired lipid product [24,77]. This stage is ideally suited to open pond systems, as the environmental stresses that stimulate production can occur naturally through the transfer of the culture from photobioreactors to the open pond.

Huntley and Redalje [77] used such a two-stage system for the production of both oil and astaxanthin (used in salmon feed) from *Haematococcus pluvialis*, and achieved an annual average microbial oil production rate >10 toe ha^{-1} per annum with a maximum rate of 24 toe ha^{-1} per annum. They also demonstrated that under similar conditions, rates of up to 76 toe ha^{-1} per annum was feasible using species with higher oil content and photosynthetic efficiency.

A conceptual two-stage oil production process was described by Rodolfi et al. [24], where 22% of the production plant was dedicated to biomass production under N-sufficient conditions, while 78% of the plant was allocated to oil production under N-deficient conditions. This would achieve lipid production equivalent to 90 kg ha^{-1} per day (10 and 80 kg ha^{-1} per day in the first and second stage, respectively). Rodolfi et al. [24] also determined that such a hybrid system could give annual lipid production rates of 20 toe ha^{-1}, and the rate could be as high as 30 toe ha^{-1} for production systems in more favourable tropical climates [24].

3.2. Heterotrophic production

Heterotrophic production has also been successfully used for algal biomass and metabolites [78,79]. In this process microalgae are grown on organic carbon substrates such as glucose in stirred tank bioreactors or fermenters. Algae growth is independent of light energy, which allows for much simpler scale-up possibilities since smaller reactor surface to volume ratio's may be used [80]. These systems provide a high degree of growth control and also lower harvesting costs due to the higher cell densities achieved [81]. The set-up costs are minimal, although the system uses more energy than the production of photosynthetic microalgae because the process cycle includes the initial production of organic carbon sources via the photosynthesis process [20].

Li et al. [82] outlined the feasibility for large-scale biodiesel production based on heterotrophic cultivation of *Chlorella protothecoides*. Other studies also suggest higher technical viability of heterotrophic production (Table 4) compared to photoautotrophic methods in either open ponds (Table 2) or closed photobioreactors (Table 3). Miao and Wu [78] also studied *C. protothecoides* and found that the lipid content in heterotrophic cells could be as high as 55%, which was 4 times higher than in autotrophic cells at 15% under similar conditions. Hence, they concluded that heterotrophic cultivation could result in higher production of biomass and accumulation of high lipid content in cells.

3.3. Mixotrophic production

Many algal organisms are capable of using either metabolism process (autotrophic or heterotrophic) for growth, meaning that they are able to photosynthesise as well as ingest prey or organic materials [83,84]. The ability of mixotrophs to process organic substrates means that cell growth is not strictly dependent on photosynthesis, therefore light energy is not an absolutely limiting factor for growth [85] as either light or organic carbon substrates can support the growth [79]. Examples of microalgae that displays mixotrophic metabolism processes for growth are the cyanobacteria *Spirulina platensis*, and the green alga *Chlamydomonas reinhardtii* [79]. The photosynthetic metabolism utilises light for growth while aerobic respiration uses an organic carbon source [84]. Growth is influenced by the media supplement with glucose during the light and dark phases, hence, there is less biomass loss during the dark phase [85].

Growth rates of mixotrophic algae (Table 5) compare favourably with cultivation of photoautotrophic algae in closed photobioreactors (Table 3). The rates are higher than for open pond cultivation (Table 2) but are considerably lower than for heterotrophic production (Table 4). Chojnacka and Noworyta [86] compared *Spirulina* sp. growth in photoautotrophic, heterotrophic and mixotrophic cultures. They found that mixotrophic cultures reduced photoinhibition and improved growth rates over both autotrophic and heterotrophic cultures. Successful production of mixotrophic algae allows the integration of both photosynthetic and heterotrophic components during the diurnal cycle. This reduces the impact of biomass loss during dark respiration and decreases the amount of organic substances utilised during growth. These features infer that that mixotrophic production can be an important part of the microalgae-to-biofuels process.

Table 5
Biomass productivity figures for microalgae mixotrophic cultures.

Species	Organic carbon source	μ_{max} (day^{-1})	X_{max} ($g\,l^{-1}$)	P_{volume} ($g\,l^{-1}\,day^{-1}$)	Reference
Spirulina platensis	Glucose	0.62	2.66	–	[79]
Spirulina platensis	Acetate	0.52	1.81	–	[79]
Spirulina sp.	Glucose	1.32	2.50	–	[86]
Spirulina platensis	Molasses	0.147	2.94	0.32	[85]

3.4. Microalgae production and biofuels productivity factors

Microalgae like other plant-based biofuel resources provide the mechanism for collection, conversion and storage of solar energy into chemical form. For biofuel production, the major factors cited as determining economically viable production include: productivity (*viz.*, strain selection, photosynthetic efficiency, and productivity of lipids), production and harvesting costs [55]. Photosynthetic efficiency is only relevant for autotrophic algae; for heterotrophically cultivated algae, the utilisation of sugars is more relevant.

3.4.1. Impact of photosynthetic efficiency (PE) on microalgal biofuel production

Photosynthetic efficiency (PE) is the fraction of light energy that is fixed as chemical energy during photoautrophic growth [87]. Only photosynthetic active radiation (PAR) of wavelengths between 400 and 700 nm, representing 42.3% of the total energy from the light spectrum is captured. The captured energy is used in the Calvin cycle to produce carbohydrates by utilising CO_2 and H_2O molecules in the process summarised by the reaction equation:

$$6CO_2 + 12H_2O + \text{photons} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2$$

A minimum of 8 light photons (quantra) is required to generate one mole of base carbohydrate (CH_2O), one O_2 molecule and one H_2 [88,89]. The average energy content of a single quantra is 218 kJ per mol; therefore, the total potential light energy captured by photosynthesis is 1744 kJ per mol of CH_2O. Given that the energy contained in one mole of CH_2O is roughly 467 kJ (one-sixth of the energy content of glucose), the efficiency of solar-to-chemical energy conversion is approximately 27%. However, since only the PAR (42.3%) can be utilised during photosynthesis process maximum PE is estimated at 11.3%. Bolton and Hall [89] calculated a theoretical maximum PE of 13% for a green-type plant in bright sunlight. This estimated value is the theoretical "upper limit" of PE, as it does not account for other factors that could decrease efficiency and conversion (e.g. photosaturation, photorespiration, and poor light absorption) and significantly reduce PE. Due to such impacting factors, most terrestrial plants attain PE levels far below the theoretical estimates, with global averages typically between 1% and 2% [88].

Their simple structure allows algae to achieve substantially higher PE values compared to terrestrial plants. For example, studies by Doucha and Lívanský [90,91] and Hase et al. [92] on *Chlorella* sp. recorded PAR-based PE values of 7.05%, 6.48% and 6.56%, respectively. *Synechococcus* sp. was found to have a PE of between 2% and 4% [93], while *Chlorella sorokiniana* with a PE of 8.66% [94] and *Chlorophyta* sp. with a PE of 4.15% [92] indicated significantly higher values for algae compared to terrestrial plants. Other studies have suggested that even higher levels of PE can be attained by microalgae [73,77,95]. For example, Hall et al. [96] and Acién Fernández et al. [97] recorded PE values of 15% and 21.6% for the microalga *Phaeodactylum tricornutum*, respectively. Other findings outperforming the base estimate used in this review include 20% PE for *Chlorella* [98], and 19% PE for *Tetraselmis suecica* [99]. Overall, the outlined evidence suggests that microalgae could be the most efficient biomass resource for biofuel production [100].

3.4.2. Impact of strain selection

The selection of appropriate algae strains is an important factor in the overall success of biofuel production from microalgae [101–103]. In the context of this review the ideal algal strain for biofuel production should: (1) have high lipid productivity; (2) be robust and able to survive the shear stresses common in photobioreactors; (3) be able to dominate wild strains in open pond production systems; (4) have high CO_2 sinking capacity; (5) have limited nutrient requirements; (6) be tolerant to a wide range in temperatures resulting from the diurnal cycle and seasonal variations; (7) provide valuable co-products; (8) have a fast productivity cycle; (9) have a high PE; and (10) display self-flocculation characteristics. At the moment, no known algal strain is capable of meeting all these requirements concurrently.

It can be argued that site specific adaptation is the key to commercial microalgae production [102]. This allows the algae to be exposed to the prevailing environmental conditions, which is a distinct advantage over imported strains [102]. de Morais and Costa [104] found that algae (*Scenedesmus obliquus* and *Chlorella kessleri*) isolated from effluent treatment ponds near a power plant have the potential for the biofixation of CO_2, although biomass productivities were lower compared to closed system production in photobioreactors. Yoo et al. [105] compared three microalgae species (*Botryococcus braunii*, *Chlorella vulgaris*, and *Scenedesmus* sp.) under high level CO_2 growth condition for biodiesel production, and concluded that function specificity is an important factor in species selection; *B. braunii* being the most suitable for biodiesel production, and *Scenedesmus* sp. being suitable for CO_2 mitigation. The isolation of local strains for biofuel production should be considered a basic research area, but it is also noteworthy that the dominant strains may not be the optimal for production of lipids, therefore genetic manipulation may be required [102].

Genetic and metabolic engineering are likely to have an impact on the performance of algal strains for biofuel production [106]. There is increasing interest in the potential of transgenic microalgae as green cell factories capable of producing both biofuels and value added products such as proteins and metabolites, but up to now this area has received little attention and is still in its infancy [107]. It has been argued that among microalgal species that colonise the photic earth zones, many organisms suitable for outdoor mass culture and biofuel production might be found. Consequently, it has been suggested that there is no apparent need to genetically modify microalgae so as to achieve the requirement for stable mass cultures with relatively high oil contents and productivity [24]. Instead it may be prudent to limit projections to what can be achieved with natural strains. Notably, the US Department of Energy's (USDOE) "Aquatic Species Program" (ASP) had collected over 3000 strains of oil-producing organisms, which after screening, isolation and characterisation efforts, the collection was narrowed down to 300 species, mostly green algae and diatoms [102].

3.4.3. Lipid productivity

While many microalgae strains naturally have high lipid content (ca. 20–50% dry weight), it is possible to increase the concentration by optimising the growth determining factors [108] such as the control of nitrogen level [109–112], light intensity [26,110], temperature [26], salinity [26,112], CO_2 concentration [40,104] and harvesting procedure [40,109]. However, increasing lipid accumulation will not result in increased lipid productivity as biomass productivity and lipid accumulation are not necessarily correlated [24,102]. Lipid accumulation refers to increased concentration of lipids within the microalgae cells without consideration of the overall biomass production. Lipid productivity takes into account both the lipid concentration within cells and the biomass produced by these cells and is therefore a more useful indicator of the potential costs of liquid biofuel production.

Initial research focused on the isolation of high lipid content microalgae that could be cultivated in large-scale open pond cultivation for biodiesel production [63,99,113–115], and capturing CO_2 from coal-fired power plants as biological emission control process [116–118]. The primary findings of the outlined research were: (1) increment in oil accumulation in algal cells due

to nitrogen-deficiency is inversely proportional to oil productivity of entire cultures due to lower total productivity resulting from lower nutrient levels; (2) open pond production is most appropriate for large-scale microalgae production due to low costs; (3) maintenance of uncontaminated mono-specific microalgae cultures in open ponds for sustainable high production is exceedingly difficult.

The most effective method of improving microalgae lipid accumulation is nitrogen limitation, which not only results in the accumulation of lipids, but also results in a gradual change of lipid composition from free fatty acids to triacylglycerol (TAG) [109]. TAGs are more useful for conversion to biodiesel [119]. Lipid accumulation in microalgae occurs when a nutrient (typically nitrogen, but can be silicate for diatoms) is exhausted from the medium or becomes the growth limiting factor. Cell proliferation is prevented but carbon is still assimilated by the cell and converted to TAG lipids that are stored within existing cells thereby increasing the concentration [119]. Wu and Hsieh [112] investigated the effects of salinity, nitrogen concentration and light intensity on lipid productivity, and recorded up to 76% increase in production of lipids for specific growth conditions when compared to more typical growth processes. Weldy and Huesemann [110] argued that for lipid production, the percentage lipid content of microalgae was less important than maximisation of growth rates. For example, they recorded higher lipids productivity (0.46 g l^{-1} per day) under N-sufficient conditions and high light intensity when compared with N-deficient cultures (0.12 g l^{-1} per day). Chiu et al. [40] established that 2% (v/v) CO_2 concentration was optimal for *Nannochloropsis oculata* to achieve maximum biomass and lipid productivity. They achieved 0.48 g l^{-1} per day and 0.142 g l^{-1} per day for biomass yield and lipid production, respectively.

4. Co-processes in microalgae production

The combined production of renewable energy and material resources with unique environmental applications for GHG emission mitigation and wastewater treatment is one of the hallmarks of microalgal research [120]. Mass cultures of microalgae have potential utilisation in the production of biofuels and chemicals, food and feed, and for CO_2 fixation and water purification [8,117,121]. These multiple applications support sustainability (key principle in natural resource management) and process economy.

4.1. Bio-mitigation of CO_2 emissions with microalgae

Microalgae can typically be used to capture CO_2 from three different sources: atmospheric CO_2, CO_2 emission from power plants and industrial processes, and CO_2 from soluble carbonate [8]. Capture of atmospheric CO_2 is probably the most basic method to sink carbon, and relies on the mass transfer from the air to the microalgae in their aquatic growth environments during photosynthesis [8]. However, the potential yield from the atmosphere is limited by low CO_2 concentration in air (360 ppm) which makes it economically infeasible [122]. In contrast, CO_2 capture from flue gas emissions from power plants that burn fossil fuels achieves better recovery due to the higher CO_2 concentration of up to 20% [7], and adaptability of this process for both photobioreactor and raceway pond systems for microalgae production. However, only a small number of algae are tolerant to the high levels of SO_x and NO_x that are present in flue gases. The gases also need to be cooled prior to injection into the growth medium. A number of microalgae species are able to assimilate CO_2 from soluble carbonates such as Na_2CO_3 and $NaHCO_3$ [8]. Due to the high salt content and resulting high pH of the medium, it is easier to control invasive species since only a very small number of algae can growth in the extreme conditions [8].

The selection of suitable microalgae strains for CO_2 bio-mitigation has significant effect on efficacy and cost competitiveness of the bio-mitigation process. The desirable attributes for high CO_2 fixation include: high growth and CO_2 utilisation rates; high tolerance of trace constituents of flue gases such as SO_x and NO_x; possibility for valuable by-products and co-products, e.g. biodiesel and biomass for solid fuels; ease of harvesting associated with spontaneous settling or bio-flocculation characteristics; high water temperature tolerance to minimise cost of cooling exhaust flue gases; be able to use the strain in conjunction with wastewater treatment. No single strain can satisfy all of the outlined requirements, but Table 6 provides data on ranges of known characteristics of selected species suitable for CO_2 mitigation.

A number of research findings have quantified the potential of microalgae for biological carbon capture under various conditions. *C. vulgaris* grown on wastewater discharge from a steel plant successfully sequestered 0.624 g CO_2 l^{-1} per day [123]. Doucha et al. [43] recorded 10–50% reduction in CO_2 concentration in flue gases using with *Chlorella* sp., with the efficacy decreasing with increasing rate of flue gas injection into microalgae culture. Their observation was corroborated by other researchers. For example,

Table 6
CO_2 and biomass productivity for CO_2 mitigation species.

Microalgae	T (°C)	CO_2 (%)	P_{volume} (g l^{-1} day^{-1})	P_{CO_2} (g l^{-1} day^{-1})	Carbon utilisation efficiency (%)	Reference
Chlorella sp.	26	Air	0.682[a]	–	–	[231]
Chlorella sp.	26	2	1.445[a]	–	58	[231]
Chlorella sp.	26	5	0.899[a]	–	27	[231]
Chlorella sp.	26	10	0.106[a]	–	20	[231]
Chlorella sp.	26	15	0.099[a]	–	16	[231]
Chlorella kessleri	30	18	0.087	–	–	[104]
Scenedesmus sp.	25	10	0.218	–	–	[105]
Chlorella vilgaris	25	10	0.105	–	–	[105]
Botryococcus braunii	25	10	0.027	–	–	[105]
Scenedesmus sp.	25	Flue gas	0.203	–	–	[105]
Botryococcus braunii	25	Flue gas	0.077	–	–	[105]
Chlorella vulgaris	25	Air	0.040	–	–	[232]
Chlorella vulgaris	25	Air	0.024	–	–	[232]
Haematococcus pluvialis	20	16–34	0.076	0.143	–	[77]
Scenedesmus obliquus	–	Air	0.009	0.016	–	[133]
Scenedesmus obliquus	–	Air	0.016	0.031	–	[133]
Chlorella vulgaris	27	15	–	0.624	–	[123]
Scenedesmus obliquus	30	18	0.14	0.260	–	[124]
Spirulina sp.	30	12	0.22	0.413	–	[124]

[a] Culture incubated for 4–8 days.

de Morais and Costa [124], using *Spirulina* sp. obtained a maximum daily CO_2 biofixation of 53.29% for 6% (v/v) CO_2 and 45.61% for 12% (v/v) CO_2 in the injected flue gas, with the highest mean fixation rate being 37.9% for 6% (v/v) CO_2. With *S. obliquus*, de Morais and Costa achieved biofixation rates of 28.08% and 13.56% for 6% (v/v) and 12% (v/v) CO_2, respectively.

Kadam [44] demonstrated the potential benefits of recycling CO_2 for microalgae biomass production through co-firing coal and microalgae to reduce the environmental impact of power generation. Their LCA results showed that co-firing reduced CO_2 and methane, hence, GHG emissions through the recycling of microalgae biomass and the reduction in coal use. They also registered lower net SO_x and NO_x particulates. de Morais and Costa [104] found the microalgae species *S. obliquus* and *C. kessleri* to be capable of growing in media containing up to 18% (v/v) CO_2. Chang and Yang [125] found that certain species of *Chlorella* could grow in an atmosphere containing CO_2 up to 40% (v/v). When comparing *B. braunii*, *C. vulgaris* and *Scenedesmus* sp. under flue gas conditions, Yoo et al. [105] found *Scenedesmus* sp. to be the most suitable for CO_2 mitigation due to high rates of biomass production (0.218 g l^{-1} per day). *B. braunii* and *Scenedesmus* sp. were found to grow better using flue gas as compared to air enhanced with CO_2. This is in line with an earlier study by Brown [33] who found that microalgae can tolerate flue gas very well.

The high cost of process technology and lack of price competitiveness of biodiesel extraction from microalgae versus petroleum diesel are key obstacles to commercial exploitation [58]. Bio-mitigation of CO_2 emissions provides a complementary function that may be exploited to reduce cost and to enable sustained utilisation of microalgae as a biofuel resource.

4.2. Waste water treatment potential of microalgae

It has been argued that biofuel production in conjunction with wastewater treatment is the area with the most plausible commercial application in the short term [126,127]. They provide a pathway for the removal of chemical and organic contaminants, heavy metals and pathogens from wastewater while producing biomass for biofuel production [128]. Savings on requirements for chemical remediation [8] and possible minimisation of fresh water use for biomass production [129] are the main drivers for production of biomass as part of a wastewater treatment process. Wastewater rich in CO_2 provides a conducive growth medium for microalgae [130] because the CO_2 balances the Redfield ratio (molecular ratio of carbon, nitrogen and phosphorus in marine organic matter, C:N:P = 106:16:1) of the wastewater allowing for faster production rates, reduced nutrient levels in the treated wastewater, decreased harvesting costs and increased lipid production [130]. However, algal wastewater treatment plants have high land requirements for open pond systems and high capital costs for photobioreactor systems.

Several applications in wastewater treatment have been reported in the literature. For example, Sawayama et al. [131] used *B. braunii* to remove nitrate and phosphate from sewage after primary treatment along with the production of hydrocarbon-rich biomass. Martínez et al. [132] achieved a significant removal of phosphorus and nitrogen from urban wastewater using the microalgal *S. obliquus*. They were able to achieve 98% elimination of phosphorus and a complete removal (100%) of ammonium in a stirred culture at 25 °C over 94 and 183 h retention time, respectively. Gomez Villa et al. [133] experimented with outdoor cultivation of microalgal *S. obliquus* in artificial wastewater, and achieved final dissolved nitrogen concentrations which were 53% and 21% of initial values in winter and summer, respectively. Phosphorus, which was only removed during the day, achieved a total reduction of 45% in the winter and 73% in the summer [133], but the relatively lower efficiencies could have been due to shorter retention times compared to the earlier study. Hodaifa et al. [134] recorded 67.4% reduction in BOD_5 with *S. obliquus* cultured in diluted (25%) industrial wastewater from olive-oil extraction. The percentage of elimination reduced to 35.5% with undiluted wastewater because of low nitrogen contents, which inhibited the microalgae growth during the exponential phase. Yun et al. [123] successfully grew *C. vulgaris* in wastewater discharge from a steel plant to achieve an ammonia bioremediation rate of 0.022 g NH_3 l^{-1} per day. To improve efficiencies, Muñoz et al. [135] found the use of a biofilm attached onto the reactor walls of flat plate and tubular photobioreactors improved BOD_5 removal rates by 19% and 40%, respectively, when compared with a control suspended bioreactor for industrial wastewater effluent. The retention of algal biomass showed remarkable potential in maintaining optimum microbial activity while remediating the effluent.

For processing of hazardous or toxic compounds, it is possible to use microalgae to generate the oxygen required by bacteria to biodegrade pollutants such as polycyclic aromatic hydrocarbons (PAHs), phenolics and organic solvents [128]. Photosynthetic oxygen from microalgae production reduces or eliminates the need for external mechanical aeration [128]. Chojnacka et al. [136] found that *Spirulina* sp. acted as a biosorbent, thus was able to absorb heavy metal ions (Cr^{3+}, Cd^{2+}, and Cu^{2+}). Biosorption properties of microalgae depended strongly on cultivation conditions with photoautrophic species showing greater biosorption characteristics.

5. Recovery of microalgal biomass

The recovery of microalgal biomass which generally requires one or more solid–liquid separation steps is a challenging phase of the algal biomass production process [8], and accounts for 20–30% of the total costs of production according to one source [137]. The processes involved include flocculation, filtration, flotation, and centrifugal sedimentation; some of which are highly energy intensive. Low cell densities (typically in the range of 0.3–5 g l^{-1}) when there is limited light penetration, and the small size of some algal cells (typically in the range of 2–40 μm), make the recovery of biomass difficult [129].

The selection of harvesting technology is crucial to economic production of microalgal biomass [17]. A factor such as strain selection is an important consideration since certain species are much easier to harvest. For example, the cyanobacterium *Spirulina*'s long spiral shape (20–100 μm long) naturally lends itself to the relatively cost-efficient and energy-efficient micro-screen harvesting method [138].

5.1. Harvesting methods

Choice of harvesting technique is dependent on characteristics of microalgae, e.g. size, density, and the value of the target products [139]. Generally, microalgae harvesting is a two stage process, involving:

(1) Bulk harvesting—aimed at separation of biomass from the bulk suspension. The concentration factors for this operation are generally 100–800 times to reach 2–7% total solid matter. This will depend on the initial biomass concentration and technologies employed, including flocculation, flotation or gravity sedimentation.
(2) Thickening—the aim is to concentrate the slurry through techniques such as centrifugation, filtration and ultrasonic aggregation, hence, is generally a more energy intensive step than bulk harvesting.

5.1.1. Flocculation and ultrasonic aggregation

This is the first stage in the bulk harvesting process that is intended to aggregate the microalgal cells in order to increase the effective "particle" size. Flocculation is a preparatory step prior to other harvesting methods such as filtration, flotation or gravity sedimentation [140]. Since microalgae cells carry a negative charge that prevents natural aggregation of cells in suspension, addition of flocculants such as multivalent cations and cationic polymers neutralises or reduces the negative charge. It may also physically link one or more particles through a process called bridging, to facilitate the aggregation [140]. Multivalent metal salts like ferric chloride ($FeCl_3$), aluminium sulphate ($Al_2(SO_4)_3$) and ferric sulphate ($Fe_2(SO_4)_3$) are suitable flocculants.

Several flocculation harvesting methods have been tested. Knuckey et al. [141] developed a process that entailed adjustment of the algae culture pH to between 10 and 10.6 using NaOH, followed by addition of a non-ionic polymer Magnafloc LT-25. The flocculate was harvested by siphoning off surface water after a settling period, and subsequently neutralised to give a final biomass concentration of 6–7 g l^{-1}. The process was successfully applied to a range of species with flocculation efficiencies of >80%. Divakaran and Pillai [142] successfully used Chitosan as a bio-flocculant. The efficacy of the method was very sensitive to pH; registering maximum flocculation at pH 7.0 for the freshwater species, and lower for the marine species. The residual water could be reused to produce fresh algae cultures.

Gentle, acoustically induced aggregation followed by enhanced sedimentation can also be used to harvest microalgae biomass. Bosma et al. [143] successfully used ultrasound to optimise the aggregation efficiency and concentration factor. They achieved 92% separation efficiency and a concentration factor of 20 times (the factor by which the original liquid mixture has been concentrated). The main advantages of ultrasonic harvesting are that it can be operated continuously without inducing shear stress on the biomass, which could destroy potentially valuable metabolites, and it is a non-fouling technique [143]. Successful applications in the medical sector [144] provides basis for further investigations on potential applications in algal biomass harvesting.

5.1.2. Harvesting by flotation

Flotation methods are based on the trapping of algae cells using dispersed micro-air bubbles and therefore, unlike flocculation, does not require any addition of chemicals [8]. Some strains naturally float at the surface of the water as the microalgal lipid content increase [103]. Although flotation has been mentioned as a potential harvesting method, there is very limited evidence of its technical or economic viability.

5.1.3. Gravity and centrifugal sedimentation

Gravity and centrifugation sedimentation methods are based on Stoke's Law [17], i.e. settling characteristics of suspended solids is determined by density and radius of algae cells (Stoke's radius) and sedimentation velocity. Gravity sedimentation is the most common harvesting technique for algae biomass in waste-water treatment because of the large volumes treated and the low value of the biomass generated [145]. However, the method is only suitable for large (ca. >70 μm) microalgae such as *Spirulina* [128].

Centrifugation recovery (CR) is preferred for harvesting of high-value metabolites and extended shelf-life concentrates for hatcheries and nurseries in aquaculture [146]. The process is rapid and energy intensive; biomass recovery depends on the settling characteristics of the cells, slurry residence time in the centrifuge, and settling depth [140]. The disadvantages of the process include high energy costs and potentially higher maintenance requirements due to freely moving parts [143]. Harvesting efficiency of >95% [146], and increase in slurry concentration by up to 150 times for 15% total suspended solids are technically feasible [147].

5.1.4. Biomass filtration

A conventional filtration process is most appropriate for harvesting of relatively large (>70 μm) microalgae such as *Coelastrum* and *Spirulina*. It cannot be used to harvest algae species approaching bacterial dimensions (<30 μm) like *Scenedesmus*, *Dunaliella* and *Chlorella* [147]. Conventional filtration operates under pressure or suction, filtration aids such as diatomaceous earth or cellulose can be used to improve efficiency [140]. Mohn [147] demonstrated that filtration processes can achieve a concentration factor of 245 times the original concentration for *Coelastrum proboscideum* to produce a sludge with 27% solids.

For recovery of smaller algae cells (<30 μm), membrane microfiltration and ultra-filtration (a form of membrane filtration using hydrostatic pressure) are technically viable alternatives to conventional filtration [148]. It is suitable for fragile cells that require low trans-membrane pressure and low cross-flow velocity conditions [53]. For processing of low broth volumes (<2 m^3 per day), membrane filtration can be more cost effective compared to centrifugation. Owing to the cost for membrane replacement and pumping in larger scales of production (>20 m^3 per day), centrifugation may be a more economic method of harvesting the biomass [149].

5.2. Extraction and purification of microalgal biomass

5.2.1. Dehydration processes

The harvested biomass slurry (typical 5–15% dry solid content) is perishable and must be processed rapidly after harvest; dehydration or drying is commonly used to extend the viability depending on the final product required. Methods that have been used include sun drying [150], low-pressure shelf drying [150], spray drying [151], drum drying [150], fluidised bed drying [152], freeze drying [153], and Refractance Window^{TM} technology drying [154].

Sun drying is the cheapest dehydration method; but main disadvantages include long drying times, the requirement for large drying surfaces, and the risk of material loss [150]. Spray drying is commonly used for extraction of high value products, but it is relatively expensive and can cause significant deterioration of some algal pigments [151]. Freeze drying is equally expensive, especially for large scale operations, but it eases extraction of oils. Intracellular elements such as oils are difficult to extract from wet biomass with solvents without cell disruption, but are extracted more easily from freeze dried biomass [140,153].

5.2.2. Extraction and purification of biofuels

For the extraction of biofuels, it is important to establish a balance between the drying efficiency and cost-effectiveness in order to maximise the net energy output of the fuels [129]. The cost of drying is also an important consideration in the processing of microalgal biomass powder for the food and feed industry [129]. Drying temperature during lipid extraction affects both the lipid composition and the lipid yield from the algal biomass [109]. For example, drying at 60 °C still retains a high concentration of TAG in the lipids and only decreases slightly the lipid yield, with higher temperatures decreasing both the concentration of TAG and lipid yield [109]. OriginOil (a biofuel company based in Los Angeles) developed a wet extraction process that combines ultrasound and electromagnetic pulse induction to break the algae cell walls. Carbon dioxide is added to the algae solution, which lowers the pH, and separates the biomass from the oil [155].

5.2.3. Extraction and purification for algal metabolites

Cell disruption is often required for recovering intracellular products from microalgae. Cell walls can strongly modulate any extraction process by reducing the cell biodegradability [156]. Most cell disruption methods applicable to microalgae have been adapted from applications on intracellular non-photosynthetic bioproducts [157]. Cell disruption methods that have been used successfully [158] include high-pressure homogenisers, autoclaving, and addition of hydrochloric acid, sodium hydroxide, or alkaline lysis.

Solvents are widely used to extract metabolites such as astaxanthin, β-carotene and fatty acids from algal biomass [140]. The process entails cell uptake of solvent molecules on exposure to a solvent, which causes alterations to the cell membrane to enhance the movement of globules toward the outside of the cell [159]. Properties of the cell membrane play an important part in solvent extraction process. For example, the presence of a cell wall may prevent direct contact between the solvent and the cell membrane and impede the extraction. Physiological properties such as the location and process by which the desirable contents accumulate in the cell can also impact on the efficacy of the solvent [159].

6. Algal biofuels conversion technologies

In this section, the technically viable conversion options for algal biomass and end-use of derived energy or energy carriers (liquid or gaseous fuels) are considered. The conversion of algal biomass-to-energy encompasses the different processes ordinarily used for terrestrial biomass and which depend, to a large extent, on the types and sources of biomass, conservation options and end-use [160]. The conversion technologies for utilising microalgae biomass can be separated into two basic categories of thermochemical and biochemical conversion (Fig. 3). Factors that influence choice of conversion process include: the type and quantity of biomass feedstock; the desired form of the energy; economic consideration; project specific; and the desired end form of the product [161].

6.1. Thermochemical conversion

Thermochemical conversion covers the thermal decomposition of organic components in biomass to yield fuel products, and is achievable by different processes such as direct combustion, gasification, thermochemical liquefaction, and pyrolysis [162]. Table 7 is a compilation of thermochemical conversion process's considered for microalgae. It shows the range of oil yields achieved and their relevant Higher Heating Value (HHV).

6.1.1. Gasification

Gasification involves the partial oxidation of biomass into a combustible gas mixture at high temperatures (800–1000 °C) [163]. In the normal gasification process, the biomass reacts with oxygen and water (steam) to generate syngas, a mixture of CO, H_2, CO_2, N, and CH_4 [164]. The key advantage of gasification as a biomass-to-energy pathway is that it can produce a syngas from a wide variety of potential feedstocks [163]. Syngas is a low calorific gas (typical 4–6 MJ m^{-3}) that can be burnt directly or used as a fuel for gas engines or gas turbines [165].

Gasification characteristics of microalgae biomass have been studied by several researchers. Hirano et al. [31] partially oxidised *Spirulina* at temperature ranging from 850 to 1000 °C, and determined the gas composition required to generate theoretical yield of methanol. They estimated that algae biomass gasification at 1000 °C produced the highest theoretical yield of 0.64 g methanol from 1 g of biomass. They estimated an energy balance (ratio of methanol produced to the total required energy) of 1.1, which gives gasification a marginal positive energy balance, the low value being attributed to the use of an energy intensive centrifuge process during biomass harvesting. Minowa and Sawayama [166] gasified the microalgae *C. vulgaris* in a novel system with nitrogen cycling to obtain methane-rich fuel with all

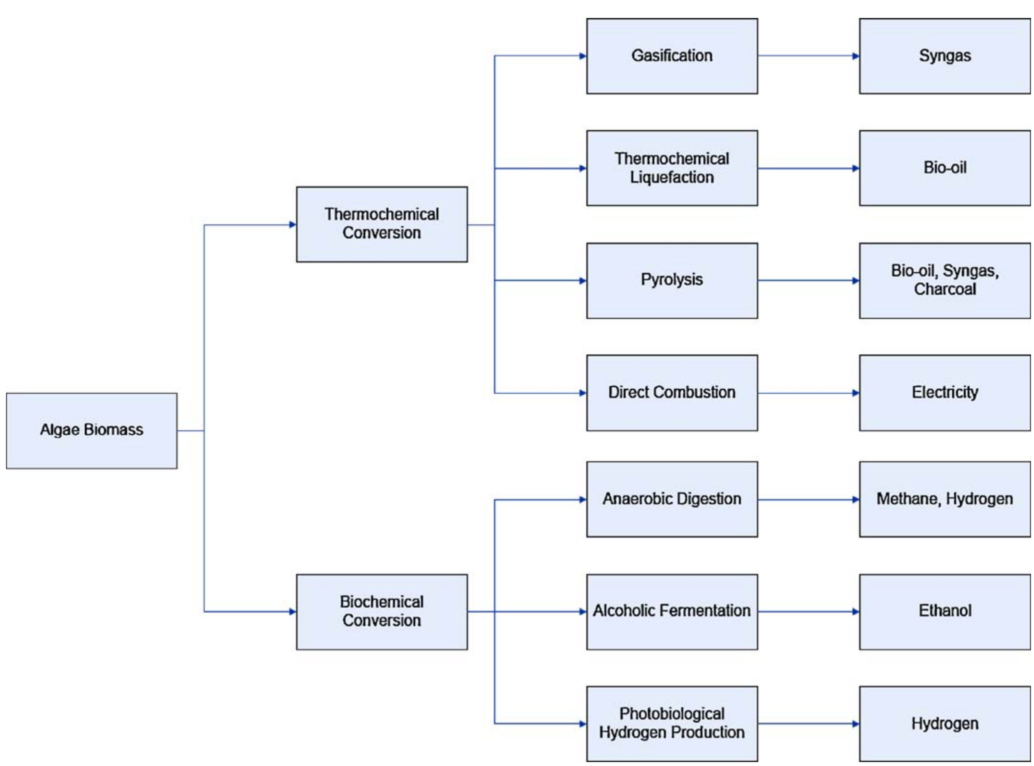


Fig. 3. Potential algal biomass conversion processes (adapted from Tsukahara and Sawayama [162]).

Table 7
Comparison between thermochemical conversion technologies.

Conversion process	Microalgae	Production	Temperature (°C)	Pressure (MPa)	Liquid		Gas	Solid	Reference
					Content (% dry wt.)	HHV (MJ kg^{-1})	Content (% dry wt.)	Content (% dry wt.)	
Gasification	*Spirulina*	N/A	1000	0.101	–	–	64	–	[31]
Thermochemical liquefaction	*Botryococcus braunii*	N/A	300	3	64	45.9	–	–	[169]
Thermochemical liquefaction	*Dunaliella tertiolecta*	N/A	300	3	42	34.9	–	–	[100]
Pyrolysis	*Chlorella prothothecoides*	Heterotrophic	450	0.101	57.9	41	32	10.1	[173]
Pyrolysis	*Chlorella prothothecoides*	Phototrophic	450	0.101	16.6	30	–	–	[173]
Pyrolysis	*Chlorella prothothecoides*	Phototrophic	500	0.101	18	30	–	–	[174]
Pyrolysis	*Chlorella prothothecoides*	N/A	502	0.101	55.3	39.7	36.3	8.4	[175]
Pyrolysis	*Microcystis aeruginosa*	Phototrophic	500	0.101	24	29	–	–	[174]

the nitrogen component of the microalgae converted into fertilizer quality ammonia.

Reliable literature data for the gasification of microalgae is very sparse, which is indicated by the lack of gasification data in Table 7. This area needs more research especially into the energy balance of drying the biomass for gasification.

6.1.2. Thermochemical liquefaction

Thermochemical liquefaction is a process that can be employed to convert wet algal biomass material into liquid fuel [167]. Thermochemical liquefaction is a low-temperature (300–350 °C), high pressure (5–20 MPa) process aided by a catalyst in the presence of hydrogen to yield bio-oil [168]. Reactors for thermochemical liquefaction and fuel-feed systems are complex and therefore expensive [160], but have advantages in their ability to convert wet biomass into energy [163]. The process utilises the high water activity in sub-critical conditions to decompose biomass materials down to shorter and smaller molecular materials with a higher energy density [167].

Several studies have investigated the characteristics of algal biomass as a feedstock (Table 7). Dote et al. [169] successfully used thermochemical liquefaction at 300 °C on *B. braunii* to achieve a maximum yield of 64% dry wt. basis of oil with HHV of 45.9 MJ kg^{-1} and also declared a positive energy balance for the process (output/input ratio of 6.67:1). In a similar study, an oil yield of 42% dry wt. was obtained from *Dunaliella tertiolecta* giving a HHV of 34.9 MJ kg^{-1} and positive energy balance of 2.94:1 [100]. These results indicate that thermochemical liquefaction is a viable option for the conversion of algal biomass-to-liquid fuel.

6.1.3. Pyrolysis

Pyrolysis is the conversion of biomass to bio-oil, syngas and charcoal at medium to high temperatures (350–700 °C) in the absence of air [168]. For biomass-to-liquid fuel conversion, it is deemed to have the potential for large scale production of biofuels that could replace petroleum based liquid fuel [170]. Table 8 outlines the characteristics and expected yields of different modes of pyrolysis [171]. Flash pyrolysis (moderate temperature (500 °C), short hot vapour residence time (about 1 s)) is deemed to be viable technique for future replacement of fossil-fuels with biomass derived liquid fuels [163] mainly because of the high biomass-to-liquid conversion ratio (95.5%) that can be achieved [170]. However, there are technical challenges as pyrolysis oils are acidic, unstable, viscous, and contain solids and chemically dissolved water [172]. Therefore, the process oil will require upgrading hydrogenation and catalytic cracking to lower oxygen content and remove alkalis [164].

Compared to other conversion technologies, research on pyrolysis of algal biomass is quite extensive and has achieved reliable and promising outcomes that could lead to commercial exploitation (Table 7). Miao and Wu [173] used fast pyrolysis to enhance oil yield from microalgae *Chlorella prothothecoides* after manipulating its metabolic pathway towards heterotrophic growth (see Section 3.2). The recorded oil yield of 57.9% dry wt. basis from heterotrophic cultivation (HHV of 41 MJ kg^{-1}) was 3.4 times higher than achieved by phototrophic cultivation and the results suggest that pyrolysis has potential in algal biomass-to-liquid conversion. Miao et al. [174] achieved bio-oil yields of 18% (HHV of 30 MJ kg^{-1}) and 24% (HHV of 29 MJ kg^{-1}) with fast pyrolysis of *C. prothothecoides* and *Microcystis aeruginosa* grown phototrophically, respectively. Demirbas [175] experimenting with *C. prothothecoides*, showed that bio-oil yield increased in line with temperature increases up to a point and then decreased at higher temperatures. For example, the yield rose from 5.7% to 55.3% with an increase from 254 to 502 °C, and subsequently decreased to 51.8% at 602 °C. They recorded a HHV from microalgae of 39.7 MJ kg^{-1} obtained at temperatures ranging from 502 to 552 °C. Results indicate that bio-oils from microalgae (Table 9) are of a higher quality than those extracted from lignocellulosic materials [174,175].

6.1.4. Direct combustion

In a direct combustion process, biomass is burnt in the presence of air to convert the stored chemical energy in biomass into hot gases [168], usually in a furnace, boiler, or steam turbine at temperatures above 800 °C. It is possible to burn any type of biomass, but combustion is only feasible for biomass with moisture content <50% dry weight [160]. The heat produced must be used immediately as storage is not a viable option [163]. Combustion of biomass for heat, power, and steam ranges from very small scale utilities (domestic space and water heating) up to large-scale industrial processes in the range of 100–300 MW [160].

Energy conversion by direct biomass combustion has the disadvantage of biomass generally requiring pre-treatment processes such as drying, chopping and grinding which incur additional energy demand, and therefore cost [168]. Conversion efficiency in large biomass-to-energy plants compares favourably to that of coal-fired power plants, but may incur higher cost due to

Table 8
Operating parameters and expected yields for pyrolysis processes [171].

Mode	Conditions	Liquid (%)	Char (%)	Gas (%)
Flash pyrolysis	Moderate temperature (500 °C), short hot vapour residence time (about 1 s)	75	2	13
Fast pyrolysis	Moderate temperature (500 °C), moderate hot vapour residence time (about 10–20 s)	50	20	30
Slow pyrolysis	Low temperature (400 °C), very long solids residence time	30	35	35

Table 9
Comparison of typical properties of petroleum oil and bio-oils from fast pyrolysis of wood and microalgae (adapted from [173,174]).

Properties	Typical values		
	Bio-oils		Petroleum oil
	Wood	Microalgae	
C (%)	56.4	62.07	83.0–87.0
H (%)	6.2	8.76	10.0–14.0
O (%)	37.3	11.24	0.05–1.5
N (%)	0.1	9.74	0.01–0.7
Density ($kg\,l^{-1}$)	1.2	1.06	0.75–1.0
Viscosity (Pa s)	0.04–0.20 (at 40 °C)	0.10 (at 40 °C)	2–1000
HHV ($MJ\,kg^{-1}$)	21	29–45.9	42

high moisture content of biomass. Generation of combined heat and power (CHP) is desirable to improve on overall plant efficiency. Net energy conversion efficiencies for biomass combustion power plants range from 20% to 40%, with higher efficiencies obtained in larger systems (>100 MW) or when biomass is co-combusted in coal fired power plants [164].

There is little evidence of technically viable utilisation of algal biomass in direct combustion in literature, but a life cycle assessment (LCA) of coal-algae co-firing [44] suggested that coal-algae co-firing could lead to lower GHG emissions and air pollution. Due to the limited data, this area will require further research to determine viability.

6.2. Biochemical conversion

The biological process of energy conversion of biomass into other fuels includes anaerobic digestion, alcoholic fermentation and photobiological hydrogen production [176].

6.2.1. Anaerobic digestion

Anaerobic digestion (AD) is the conversion of organic wastes into a biogas, which consists of primarily methane (CH_4) and carbon dioxide, with traces of other gases such as hydrogen sulphide [177]. It involves the breakdown of organic matter to produce a gas with an energy content of about 20–40% of the lower heating value of the feedstock. Anaerobic digestion process is appropriate for high moisture content (80–90% moisture) organic wastes [160], which can be useful for wet algal biomass.

The AD process occurs in three sequential stages of hydrolysis, fermentation and methanogenesis. In hydrolysis the complex compounds are broken down into soluble sugars. Then, fermentative bacteria convert these into alcohols, acetic acid, volatile fatty acids (VFAs), and a gas containing H_2 and CO_2, which is metabolised into primarily CH_4 (60–70%) and CO_2 (30–40%) by methanogens [23]. It has been estimated that the conversion of algal biomass into methane could recover as much energy as obtained from the extraction of cell lipids [156], while leaving a nutrient rich waste product that can be recycled into a new algal growth medium [178,179].

Microalgae can have a high proportion of proteins that result in low C/N ratios (ca. 10) which can affect the performance of the anaerobic digester. This problem may be resolved by co-digestion with a high C/N ratio product (e.g. waste paper). Yen and Brune [180] achieved a significant increase in methane production with the addition of waste paper to algal biomass. They obtained double the methane production rate (1.17 $ml\,l^{-1}$ per day vs. 0.57 $ml\,l^{-1}$ per day) from 50/50 waste paper/algal biomass blend compared to anaerobic digestion of pure algal biomass. High protein content in the algae can also result in increased ammonium production, which inhibit anaerobic microorganisms. Also, sodium ions can prove toxic to some anaerobic microorganisms, but it is feasible to use salt-adapted microorganisms for the anaerobic digestion of marine algae biomass.

6.2.2. Alcoholic fermentation

Alcoholic fermentation is the conversion of biomass materials which contain sugars, starch or cellulose into ethanol [160]. The biomass is ground down and the starch is converted to sugars which is then mixed with water and yeast and kept warm in large tanks called fermenters [164]. The yeast breaks down the sugar and converts it to ethanol [160]. A purification process (distillation) is required to remove the water and other impurities in the diluted alcohol product (10–15% ethanol). The concentrated ethanol (95% volume for one distillation) is drawn off and condensed into liquid form, which can be used as a supplement or substitute for petrol in cars [164]. The solid residue from the process can be used for cattle-feed or for gasification [160]. This helps offset feedstock costs which typically make up 55–80% of the final alcohol selling price. Starch based biomass like microalgae require additional processing before fermentation [164].

Microalgae such as *C. vulgaris* are a good source of ethanol due to the high starch content (ca. 37% dry wt.), and for which up to 65% ethanol conversion efficiency has been recorded [25]. Ueno et al. [181] also produced ethanol from microalgae via dark fermentation process and achieved a maximum ethanol productivity of 450 $\mu mol\,g^{-1}$ dry wt. at 30 °C. From the outlined concepts it is arguable that ethanol production from microalgae is technically viable. However, in this review, microalgae potential is analysed in the context of lipid production, and ethanol production is treated as a conversion pathway for the waste algae biomass from oil extraction.

6.2.3. Photobiological hydrogen production

Hydrogen (H_2) is a naturally occurring molecule, which is a clean and efficient energy carrier [163]. Microalgae possess the necessary genetic, metabolic and enzymatic characteristics to photoproduce H_2 gas [27]. Under anaerobic conditions hydrogen is produced from eukaryotic microalgae either as an electron donor in the CO_2 fixation process or evolved in both light and dark [182]. During photosynthesis, microalgae convert water molecules into hydrogen ions (H^+) and oxygen; the hydrogen ions are then subsequently converted by hydrogenase enzymes into H_2 under anaerobic conditions [23]. Due to reversibility of the reaction, hydrogen is either produced or consumed by the simple conversion of protons to hydrogen [163]. Photosynthetic oxygen production causes rapid inhibition to the key enzyme, hydrogenase, and the photosynthetic hydrogen production process is impeded [23,87,183–185]. Consequently, microalgae cultures for hydrogen production must be subjected to anaerobic conditions.

There are two fundamental approaches for photosynthetic H_2 production from water. The first H_2 production process is a two-stage photosynthesis process where photosynthetic oxygen production and H_2 gas generation are spatially separated [27]. In the first stage, algae are grown photosynthetically in normal conditions. During the second stage, the algae are deprived of sulphur thereby inducing anaerobic conditions and stimulating consistent hydrogen production [186]. This production process becomes limited with time, as hydrogen yield will begin to level off after 60 h of production. The use of this production system does not generate toxic or environmentally harmful products but could give value added products as a result of biomass cultivation [185].

The second approach involves the simultaneous production of photosynthetic oxygen and H_2 gas. In this approach, electrons that are released upon photosynthetic H_2O oxidation are fed directly into the hydrogenase-mediated H_2-evolution process [27]. The H_2 productivity is theoretically superior to the two-stage photosynthetic process, but the simultaneous production process suffers

Table 10
Selected properties of 1st generation biodiesel, algal bio-oil and typical no. 2 diesel [174,233–235].

Fuel property	1st generation biodiesel	Algal biodiesel	Diesel	EN14214 biodiesel standard
HHV ($MJ\,kg^{-1}$)	31.8–42.3	41	45.9	–
Kinematic viscosity ($mm^2\,s^{-1}$)	3.6–9.48	5.2	1.2–3.5	3.5–5.2
Density ($kg\,l^{-1}$)	0.86–0.895	0.864	0.83–0.84	0.86–0.90
Carbon (wt%)	77	–	87	–
Hydrogen (wt%)	12	–	13	–
Oxygen (wt%)	11	–	0	–
Sulphur (wt%)	0.0–0.0015	–	0.05 max	<10
Boiling point (°C)	315–350	–	180–340	–
Flash point (°C)	100–170	115	60–80	>101
Cloud point (°C)	−3 to 12	–	−15 to 5	–
Pour point (°C)	−15 to 10	−12	−35 to −15	–
Cetane number	45–65	–	51	>51

severe hydrogenase inhibition after a very short period due to the photosynthetic production of oxygen [27]. Melis and Happe [186] found that using the two-stage photosynthesis process and H_2 production a theoretical maximum yield of hydrogen by green algae could be about 198 kg H_2 ha^{-1} per day.

6.3. Algal biomass-to-biodiesel

Biodiesel is a derivative of oilcrops and biomass which can be used directly in conventional diesel engines [163]. It is a mixture of monoalkyl esters of long chain fatty acids (FAME) derived from a renewable lipid feedstock such as algal oil [187]. After the extraction processes (see Section 5), the resulting product algal oil can be converted into biodiesel through a process called transesterification. Transesterification is a chemical reaction between triglycerides and alcohol in the presence of a catalyst to produce mono-esters that are termed as biodiesel [188].

For algal biodiesel to be an accepted substitution fuel for fossil fuels, its properties must match or exceed the International Biodiesel Standard for Vehicles (EN14214). Algal oils contain a high degree of polyunsaturated fatty acids when compared to vegetable oils, which makes it susceptible to oxidation in storage and therefore limits utilisation [20]. Nevertheless, algal biodiesel has similar physical and chemical properties to petroleum diesel, 1st generation biodiesel from oil crops and compares favourably with the international standard EN14214 (Table 10).

Algal biodiesel has several advantages over petroleum diesel in that: it is derived from biomass and therefore is renewable, biodegradable, and quasi-carbon neutral under sustainable production; it is non-toxic and contains reduced levels of particulates, carbon monoxide, soot, hydrocarbons and SO_x. It must be noted that compared to 1st generation biodiesel, algal biodiesel is more suitable for use in the aviation industry where low freezing points and high energy densities are key criteria [189]. Another major advantage of algal biodiesel is in reduced CO_2 emissions of up to 78% compared to emissions from petroleum diesel [190].

7. Other applications of microalgae extracts

The commercial potential for microalgae represents a largely untapped resource. It is estimated that possibly several million species of algae exist compared to around 250,000 species terrestrial plants [191]. Commercial large-scale production of microalgae started in the early 1960s in Japan with the culture of *Chlorella as a food additive*, which was followed in the 1970s and 1980s by expanded world production in countries such as USA, India, Israel, and Australia [22,29,54]. In 2004, the microalgae industry had grown to produce 7000 tonnes of dry matter per annum (Table 11) [192].

7.1. Microalgae uses in human nutrition

The human consumption of microalgae biomass is restricted to very few species due to the strict food safety regulations [192], commercial factors, market demand and specific preparation. *Chlorella*, *Spirulina* and *Dunaliella* dominate the market. Microalgae biomass is marketed in tablet or powder form as food additives generally in the health food market, which is expected to remain a

Table 11
Present state of microalgal production [22,159,192,210,236].

Microalgae	Annual production	Producer country	Application and product	Price (€)
Spirulina	3000 tonnes dry weight	China, India, USA, Myanmar, Japan	Human nutrition	$36\,kg^{-1}$
			Animal nutrition	
			Cosmetics	
			Phycobiliproteins	$11\,mg^{-1}$
Chlorella	2000 tonnes dry weight	Taiwan, Germany, Japan	Human nutrition	$36\,kg^{-1}$
			Cosmetics	
			Aquaculture	$50\,l^{-1}$
Dunaliella salina	1200 tonnes dry weight	Australia, Israel, USA, Japan	Human nutrition	
			Cosmetics	
			B-carotene	$215–2150\,kg^{-1}$
Aphanizomenon flos-aquae	500 tonnes dry weight	USA	Human nutrition	
Haematococcus pluvialis	300 tonnes dry weight	USA, India, Israel	Aquaculture	$50\,l^{-1}$
			Astaxanthin	$7150\,kg^{-1}$
Crypthecodinium cohnii	240 tonnes DHA oil	USA	DHA oil	$43\,g^{-1}$
Shizochytrium	10 tonnes DHA oil	USA	DHA oil	$43\,g^{-1}$

stable market [22]. In 2003 recorded production of *Chlorella*, which has a nutrient value and high protein content, was 2000 tonnes per annum (Table 11). *Chlorella* is also used for medicinal value such as protection against renal failure and growth promotion of intestinal lactobacillus [193]. *D. salina*, with an annual production of 1200 tonnes per annum (Table 11) is exploited for its β-carotene content of up to 14% [21].

There are health concerns over the ingestion of cyanobacteria (e.g. *Spirulina*). Cox et al. [194] studied over 50 strains of cyanobacteria and found that nearly all the strains produced the neurotoxin β-N-methylamino-L-alanine (BMAA). BMAA is linked to amyptrophic lateral sclerosis–Parkinsonism dementia complex, Lou Gehrig's disease (ALS) and Alzheimer's disease.

7.2. Microalgae uses in animal feed and aquaculture

Specific algal species are suitable for preparation of animal feed supplements. Algae species such as *Chlorella*, *Scenedesmus* and *Spirulina* have beneficial aspects including improved immune response, improved fertility, better weight control, healthier skin and a lustrous coat [192]. However, prolonged feeding at high concentrations could be detrimental [22] especially in relation to cyanobacteria. Algae are the natural food source of many important aquaculture species such as molluscs, shrimps and fish [22]. The main applications for algal biomass in aquaculture are: fish feed [195] including larval nutrition for molluscs or peneid shrimp [196]; colouring for farmed salmonids [196]; stabilisation and improvement of quality of culture medium ('green-water' technique) [197]; inducement of essential biological activities in bred aquatic species [196]; and enhancement of the immune systems of fish [192].

7.3. Microalgal applications as biofertiliser

Some conversion technologies (see Section 6), most notably pyrolysis, result in the formation of the solid charcoal residue "biochar", that has potential agricultural applications as a biofertiliser and for carbon sequestration [198–200]. Biochar can also be used as process fuel in bioenergy conversion. When applied for carbon sequestration proposes, it is considered a long-term sink that could be used to reduce carbon dioxide emissions by up to 84% [201]. It was suggested by Lehmann [201] that biochar sequestration offers the potential to produce a carbon-negative biofuel. However, the net value of GHG emission reduction due to incorporation of biochar into soils is still inconclusive [202].

7.4. Microalgae as source of polyunsaturated fatty acids

Polyunsaturated fatty acids (PUFAs) are essential for human development and physiology [203]. Among other things, PUFAs have been proven to reduce the risk of cardiovascular disease [204,205]. Currently, fish and fish oil are the main sources of PUFA but application as a food additive are limited due to possible accumulation of toxins, fish odour, unpleasant taste, poor oxidative stability, the presence of mixed fatty acids [192] and not suitable for vegetarian diets.

Microalgae are a primary source of PUFA (Table 12), and supply whole food chains with these vital components as higher plants and animals lack the requisite enzymes to synthesize PUFA [192]. Microalgal PUFA also has many other applications such as additives for infant milk formula. Elsewhere, chickens have been fed with special algae to produce omega-3 enriched eggs [192]. Currently, docosahexaenoic acid (DHA) is the only algal PUFA that is commercially available, because algal extracts are still not competitive sources of eicosapentaenoic acid (EPA), γ-linolenic acid (GLA), and arachidonic acid (AA) against other primary sources [22].

7.5. Microalgal recombinant proteins

Important recombinant protein extracts include β-carotene, astaxanthin, and C-phycocyanin (C-PC). The carotenoid β-carotene has a wide range of applications. It can be used as a food colouring agent, a source of pro-vitamin A and as a additive to cosmetics [206]. *D. salina* is the most suitable biological source of β-carotene, and can produce up to 14% dry wt. [22]. The majority (>90%) of β-carotene on the market is chemically synthesised [207] and prices for natural β-carotene range from €215 to €2150 per kilogram [22].

The carotenoid astaxanthin has potential applications in the nutraceuticals, cosmetics, food and feed industries [208]. It is a potent antioxidant [209] and has possible roles in human health such as UV-light protection, immune enhancement, hormone precursor, pro-vitamin A source and for anti-inflammation [210]. It is also a strong colouring agent, with uses for colouring muscles in fish [192]. The microalgae *H. pluvialis* is a rich natural source of astaxanthin [208], able to produce 1–8% astaxanthin dry wt. [159]. Natural astaxanthin is preferred over synthetic astaxanthin due to enhanced deposition of natural pigments, regulatory requirements and consumer demand for natural products [22]. The market value for natural astaxanthin is €7150 per kilogram [101].

C-phycocyanin (C-PC) is a major photosynthetic blue pigment found in cyanobacteria, rhodophytes and cryptophytes [80] and belongs to a group of light harvesting proteins called phycobiliproteins. C-PC has applications as a nutrient for both humans and animals, as a natural dye for food and cosmetics and in the pharmaceutical industry due to its antioxidative properties [79]. Currently, C-PC is used as an ingredient in cyanobacterial based foods and health foods [80]. Its primary potential seems to be as a natural dye, replacing current synthetic pigments [22]. However, recent research and developments have expanded the potential applications of C-PC in biotechnology, diagnostics, foods and medicine [80]. For example, their properties make them very powerful and highly sensitive fluorescent reagents where they can be used in immunolabelling experiments as labels for antibodies, receptors and other biological molecules [211]. It is currently extracted from open pond cultures of the cyanobacteria *Spirulina* (*Arthrospira*) *platensis* [212,213] and the rhodophyte (red algae) *Porphyridium cruentum* [211]. The prices of phycobiliprotein products ranges from €215 to €1790 per kilogram for native pigment but can reach €10,700 per kilogram for cross-linked pigments [22].

Table 12
Potential of microalgae as primary PUFA resources [22].

PUFA	Potential application	Microalgal producer
Docosahexaenoic acid (DHA)	Infant formulas; Nutritional supplements; Aquaculture	*Crypthecodinium, Schizochytrium*
Eicosapentaenoic acid (EPA)	Nutritional supplements; Aquaculture	*Nannochloropsis, Phaeodactylum, Nitzschia, Pavlova*
γ-Linolenic acid (GLA)	Infant formulas; Nutritional supplements	*Spirulina*
Arachidonic acid (AA)	Infant formulas; Nutritional supplements	*Porphyridium*

8. Conclusions

This review underlines the existing technical viability for the development of biofuels from microalgae as a renewable energy resource and for mitigation of GHG related impacts of petroleum derived fuels. The achievable high yields for both lipids and biomass, combined with some useful co-products if purposefully exploited, could enhance algae's economic viability as a source for biofuels.

Phototrophic production is the most effective in terms of net energy balance. However, productivity values vary immensely and are significantly lower when compared with heterotrophic production. Overall, the technical viability of a production system hinges on the intrinsic properties of the selected algae strain, indicating a need for greater species screening, as well as research on culture conditions and production systems. Bio-mitigation of CO_2 emissions with microalgae provides a complementary function that may be exploited to moderate the cost of biofuels production. The use of waste CO_2 from power plants to enhance production has been shown to be technically feasible, and hence, may be deployed to reduce production costs and for GHG emission control.

Harvesting of algal biomass accounts for the highest proportion of energy input during production, but currently, there are no standard harvesting techniques. Adaptation of technologies already in use in the food, biopharmaceutical and wastewater treatment sector may provide possible solutions. Lipids are the most readily extractible biofuel feedstock from algae, but potential storage is hindered by the presence of polyunsaturated fatty acids (PUFAs) causing oxidation reactions and high moisture content of algal feedstock. This review also suggests that both thermochemical liquefaction and pyrolysis appear to be the most technically feasible methods for conversion of algal biomass-to-biofuels, after the extraction of oils from algae.

Evidence in this review suggests that the concurrent extraction of valuable co-products (*viz.*, β-carotene, PUFA, biofertilisers, among others) with biofuel production has significant potential. Therefore, large-scale production of microalgae for biofuels will increase the availability of these products. Overall, with the current demand for renewable fuels, especially for use in the transportation sector, there is a need to develop a range of sustainable biofuels resources as the combined mix will be a significant step towards the replacement of fossil fuels. Continued development of technologies to optimise the microalgae production, oil extraction and biomass processing has the capacity to make significant contributions towards this goal.

Acknowledgement

This study was funded under the Charles Parsons Energy Research Programme of Science Foundation Ireland (SFI).

References

[1] BP. BP statistical review of world energy; 2009.
[2] European Commission. Communication from the commission to the European council and the European parliament: an energy policy for Europe. In: EC COM(2007) 1 Final; 2007. p. 27..
[3] Rogner HH. Energy resources. In: Goldemberg J, editor. World energy assessment: energy and the challenge of sustainability. New York: UNDP/UNDESA/WEC; 2000.
[4] Ugarte DG, Walsh ME, Shapouri H, Slinsky P. The economic impacts of bioenergy crop production in US agriculture, USDA Agricultural Economic Report No. 816; 2003. p. 41.
[5] IPCC. Climate change 2001: impacts, adaptation, and vulnerability. In: A report of working group II of the Intergovernmental Panel on Climatic Change (IPCC). Cambridge; 2001.
[6] EIA. International carbon dioxide emissions from the consumption of energy, Available from: http://www.eia.doe.gov/pub/international/iealf/tableh1co2.xls; 2006 [cited 10.02.09].
[7] Bilanovic D, Andargatchew A, Kroeger T, Shelef G. Freshwater and marine microalgae sequestering of CO_2 at different C and N concentrations—response surface methodology analysis. Energy Conversion and Management 2009;50(2):262–7.
[8] Wang B, Li Y, Wu N, Lan C. CO_2 bio-mitigation using microalgae. Applied Microbiology and Biotechnology 2008;79(5):707–18.
[9] Sorest JP. Climatic change: solutions in sight, a Dutch perspective. Delft: Energy Policy Platform; 2000.
[10] IEA. World energy outlook 2007. Paris: International Energy Agency; 2007.
[11] FAO. The state of food and agriculture 2008. New York: Food and Agriculture Organization; 2008.
[12] FAO. Sustainable bioenergy: a framework for decision makers. United Nations Energy; 2007.
[13] IEA. IEA technology essentials—biofuel production. International Energy Agency; 2007
[14] Moore A. Biofuels are dead: long live biofuels(?)—part one. New Biotechnology 2008;25(1):6–12.
[15] IEA. World energy outlook 2006. Paris: International Energy Agency; 2006.
[16] Khosla V. Where will biofuels and biomass feedstocks come from? [White Paper], Available from: http://www.khoslaventures.com/presentations/WhereWillBiomassComeFrom.doc [11.06.08]; 2009. p. 31.
[17] Schenk P, Thomas-Hall S, Stephens E, Marx U, Mussgnug J, Posten C, et al. Second generation biofuels: high-efficiency microalgae for biodiesel production. BioEnergy Research 2008;1(1):20–43.
[18] Dismukes GC, Carrieri D, Bennette N, Ananyev GM, Posewitz MC. Aquatic phototrophs: efficient alternatives to land-based crops for biofuels. Current Opinion in Biotechnology 2008;19(3):235–40.
[19] Searchinger T, Heimlich R, Houghton RA, Dong F, Elobeid A, Fabiosa J, et al. Use of U.S. croplands for biofuels increases greenhouse gases through emissions from land-use change. Science 2008;319(5867):1238–40.
[20] Chisti Y. Biodiesel from microalgae. Biotechnology Advances 2007;25(3):294–306.
[21] Metting FB. Biodiversity and application of microalgae. Journal of Industrial Microbiology 1996;17(5–6):477–89.
[22] Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. Journal of Bioscience and Bioengineering 2006;101(2):87–96.
[23] Cantrell KB, Ducey T, Ro KS, Hunt PG. Livestock waste-to-bioenergy generation opportunities. Bioresource Technology 2008;99(17):7941–53.
[24] Rodolfi L, Zittelli GC, Bassi N, Padovani G, Biondi N, Bonini G, et al. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnology and Bioengineering 2008;102(1):100–12.
[25] Hirano A, Ueda R, Hirayama S, Ogushi Y. CO_2 fixation and ethanol production with microalgal photosynthesis and intracellular anaerobic fermentation. Energy 1997;22(2–3):137–42.
[26] Qin J. Bio-hydrocarbons from algae—impacts of temperature, light and salinity on algae growth. Barton, Australia: Rural Industries Research and Development Corporation; 2005.
[27] Ghirardi ML, Zhang L, Lee JW, Flynn T, Seibert M, Greenbaum E, et al. Microalgae: a green source of renewable H_2. Trends in Biotechnology 2000;18(12):506–11.
[28] Ono E, Cuello JL. Feasibility assessment of microalgal carbon dioxide sequestration technology with photobioreactor and solar collector. Biosystems Engineering 2006;95(4):597–606.
[29] Pulz O, Scheinbenbogan K. Photobioreactors: design and performance with respect to light energy input. Advances in Biochemical Engineering/Biotechnology 1998;59:123–52.
[30] Ugwu CU, Aoyagi H, Uchiyama H. Photobioreactors for mass cultivation of algae. Bioresource Technology 2008;99(10):4021–8.
[31] Hirano A, Hon-Nami K, Kunito S, Hada M, Ogushi Y. Temperature effect on continuous gasification of microalgal biomass: theoretical yield of methanol production and its energy balance. Catalysis Today 1998;45(1–4):399–404.
[32] Pulz O. Photobioreactors: production systems for phototrophic microorganisms. Applied Microbiology and Biotechnology 2001;57(3):287–93.
[33] Brown LM. Uptake of carbon dioxide from flue gas by microalgae. Energy Conversion and Management 1996;37(6–8):1363–7.
[34] Falkowski PG, Raven JA. Aquatic photosynthesis. London: Blackwater Science; 1997, 375.
[35] Lee RE. Phycology. New York: Cambridge University Press; 1980.
[36] Khan SA, Rashmi, Hussain MZ, Prasad S, Banerjee UC. Prospects of biodiesel production from microalgae in India. Renewable and Sustainable Energy Reviews 2009;13(9):2361–72.
[37] Zilinskas Braun G, Zilinskas Braun B. Light absorption, emission and photosynthesis. In: Stewart WDP, editor. Algal physiology and biochemistry. Oxford: Blackwell Scientific Publications; 1974.
[38] Janssen M, Tramper J, Mur LR, Wijffels RH. Enclosed outdoor photobioreactors: light regime, photosynthetic efficiency, scale-up, and future prospects. Biotechnology and Bioengineering 2003;81(2):193–210.
[39] Muller-Feuga A, Le Guédes R, Hervé A, Durand P. Comparison of artificial light photobioreactors and other production systems using *Porphyridium cruentum*. Journal of Applied Phycology 1998;10(1):83–90.

[40] Chiu S-Y, Kao C-Y, Tsai M-T, Ong S-C, Chen C-H, Lin C-S. Lipid accumulation and CO_2 utilization of *Nanochloropsis oculata* in response to CO_2 aeration. Bioresource Technology 2009;100(2):833–8.
[41] Hsueh HT, Chu H, Yu ST. A batch study on the bio-fixation of carbon dioxide in the absorbed solution from a chemical wet scrubber by hot spring and marine algae. Chemosphere 2007;66(5):878–86.
[42] Vunjak-Novakovic G, Kim Y, Wu X, Berzin I, Merchhuk JC. Air-lift bioreactors for algal growth on flue gas: mathematical modeling and pilot-plant studies. Industrial & Engineering Chemistry Research 2005;44(16):6154–63.
[43] Doucha J, Straka F, Lívanský K. Utilization of flue gas for cultivation of microalgae (*Chlorella* sp.) in an outdoor open thin-layer photobioreactor. Journal of Applied Phycology 2005;17(5):403–12.
[44] Kadam KL. Environmental implications of power generation via coal-microalgae cofiring. Energy 2002;27(10):905–22.
[45] Emma Huertas I, Colman B, Espie GS, Lubian LM. Active transport of CO_2 by three species of marine microalgae. Journal of Phycology 2000;36(2):314–20.
[46] Colman B, Rotatore C. Photosynthetic inorganic carbon uptake and accumulation in two marine diatoms. Plant Cell and Environment 1995;18(8):919–24.
[47] Suh IS, Lee CG. Photobioreactor engineering: design and performance. Biotechnology and Bioprocess Engineering 2003;8(6):313–21.
[48] Welsh DT, Bartoli M, Nizzoli D, Castaldelli G, Riou SA, Viaroli P. Denitrification, nitrogen fixation, community primary productivity and inorganic-N and oxygen fluxes in an intertidal *Zostera noltii* meadow. Marine Ecology Progress Series 2000;208:65–77.
[49] Moreno J, Vargas MÁ, Rodríguez H, Rivas J, Guerrero MG. Outdoor cultivation of a nitrogen-fixing marine cyanobacterium, *Anabaena* sp. ATCC 33047. Biomolecular Engineering 2003;20(4–6):191–7.
[50] Hsieh C-H, Wu W-T. Cultivation of microalgae for oil production with a cultivation strategy of urea limitation. Bioresource Technology 2009;100 (17):3921–6.
[51] Çelekli A, Yavuzatmaca M, Bozkurt H. Modeling of biomass production by *Spirulina platensis* as function of phosphate concentrations and pH regimes. Bioresource Technology 2009;100(14):3625–9.
[52] Martin-Jézéquel V, Hildebrand M, Brzezinski MA. Silicon metabolism in diatoms: implications for growth. Journal of Phycology 2000;36(5):821–40.
[53] Borowitzka M. Microalgae for aquaculture: opportunities and constraints. Journal of Applied Phycology 1997;9(5):393–401.
[54] Borowitzka MA. Commercial production of microalgae: ponds, tanks, tubes and fermenters. Journal of Biotechnology 1999;70(1–3):313–21.
[55] Borowitzka M. Algal biotechnology products and processes—matching science and economics. Journal of Applied Phycology 1992;4(3):267–79.
[56] Jiménez C, Cossío BR, Labella D, Xavier Niell F. The feasibility of industrial production of *Spirulina* (*Arthrospira*) in southern Spain. Aquaculture 2003;217(1–4):179–90.
[57] Terry KL, Raymond LP. System design for the autotrophic production of microalgae. Enzyme and Microbial Technology 1985;7(10):474–87.
[58] Chisti Y. Biodiesel from microalgae beats bioethanol. Trends in Biotechnology 2008;26(3):126–31.
[59] Tan HH. Algae-to-biodiesel at least five to 10 years away, Available from: http://www.energycurrent.com/index.php?id=3&storyid=14415;2008 [cited 12.01.09].
[60] Lee YK. Microalgal mass culture systems and methods: their limitation and potential. Journal of Applied Phycology 2001;13(4):307–15.
[61] Setlik I, Veladimir S, Malek I. Dual purpose open circulation units for large scale culture of algae in temperate zones. I. Basic design considerations and scheme of a pilot plant. Algologie Studies (Trebon) 1970;1(11).
[62] Becker EW. Microalgae. Cambridge: Cambridge University Press; 1994.
[63] Weissman JC, Tillett DM. Design and operation of outdoor microalgae test facility. In: Brown LM, Sprague S, editors. Aquatic species report; NREL/MP-232-4174. National Renewable Energy Laboratory; 1992. p. 32–57.
[64] Carvalho AP, Meireles LA, Malcata FX. Microalgal reactors: a review of enclosed system designs and performances. Biotechnology Progress 2006;22(6):1490–506.
[65] Molina Grima E, Belarbi EH, Acién Fernández FG, Robles Medina A, Chisti Y. Tubular photobioreactor design for algal cultures. Journal of Biotechnology 2001;92(2):113–31.
[66] Sánchez Mirón A, Contreras Gomez A, Garca Camacho F, Molina Grima E, Chisti Y. Comparative evaluation of compact photobioreactors for large-scale monoculture of microalgae. Journal of Biotechnology 1999;70(1–3):249–70.
[67] Ugwu CU, Ogbonna J, Tanaka H. Improvement of mass transfer characteristics and productivities of inclined tubular photobioreactors by installation of internal static mixers. Applied Microbiology and Biotechnology 2002;58(5):600–7.
[68] Watanabe Y, Saiki H. Development of a photobioreactor incorporating *Chlorella* sp. for removal of CO_2 in stack gas. Energy Conversion and Management 1997;38(Suppl. 1):S499–503.
[69] Eriksen N. The technology of microalgal culturing. Biotechnology Letters 2008;30(9):1525–36.
[70] Samson R, Leduy A. Multistage continuous cultivation of blue-green alga *Spirulina maxima* in flat tank photobioreactors. Canadian Journal of Chemical Engineering 1985;63:105–12.
[71] Hu Q, Kurano N, Kawachi M, Iwasaki I, Miyachi A. Ultrahigh-cell-density culture of a marine alga *Chlorococcum littorale* in a flat-plate photobioreactor. Applied Microbiology and Biotechnology 1998;46:655–62.
[72] Richmond A, Cheng-Wu Z, Zarmi Y. Efficient use of strong light for high photosynthetic productivity: interrelationships between the optical path, the optimal population density and cell-growth inhibition. Biomolecular Engineering 2003;20(4–6):229–36.
[73] Richmond A. Microalgal biotechnology at the turn of the millennium: a personal view. Journal of Applied Phycology 2000;12(3–5):441–51.
[74] Olaizola M. Commercial production of astaxanthin from *Haematococcus pluvialis* using 25,000-liter outdoor photobioreactors. Journal of Applied Phycology 2000;12(3):499–506.
[75] Suh IS, Lee SB. A light distribution model for an internally radiating photobioreactor. Biotechnology and Bioengineering 2003;82:180–9.
[76] Sánchez Mirón A, Ceron Garcia M-C, Garcia Camacho F, Molina Grima E, Chisti Y. Growth and biochemical characterization of microalgal biomass produced in bubble column and airlift photobioreactors: studies in fed-batch culture. Enzyme and Microbial Technology 2002;31(7):1015–23.
[77] Huntley M, Redalje D. CO_2 mitigation and renewable oil from photosynthetic microbes: a new appraisal. Mitigation and Adaptation Strategies for Global Change 2007;12(4):573–608.
[78] Miao X, Wu Q. Biodiesel production from heterotrophic microalgal oil. Bioresource Technology 2006;97(6):841–6.
[79] Chen F, Zhang Y, Guo S. Growth and phycocyanin formation of *Spirulina platensis* in photoheterotrophic culture. Biotechnology Letters 1996;18(5):603–8.
[80] Eriksen N. Production of phycocyanin—a pigment with applications in biology, biotechnology, foods and medicine. Applied Microbiology and Biotechnology 2008;80(1):1–14.
[81] Chen G-Q, Chen F. Growing phototrophic cells without light. Biotechnology Letters 2006;28(9):607–16.
[82] Li X, Xu H, Wu Q. Large-scale biodiesel production from microalga *Chlorella protothecoides* through heterotrophic cultivation in bioreactors. Biotechnology and Bioengineering 2007;98(4):764–71.
[83] Graham LE, Graham JM, Wilcox LW. Algae, 2nd ed., San Francisco: Pearson Education, Inc.; 2009.
[84] Zhang XW, Zhang YM, Chen F. Application of mathematical models to the determination optimal glucose concentration and light intensity for mixotrophic culture of *Spirulina platensis*. Process Biochemistry 1999;34(5): 477–81.
[85] Andrade MR, Costa JAV. Mixotrophic cultivation of microalga *Spirulina platensis* using molasses as organic substrate. Aquaculture 2007;264(1–4): 130–4.
[86] Chojnacka K, Noworyta A. Evaluation of *Spirulina* sp. growth in photoautotrophic, heterotrophic and mixotrophic cultures. Enzyme and Microbial Technology 2004;34(5):461–5.
[87] Akkerman I, Janssen M, Rocha J, Wijffels RH. Photobiological hydrogen production: photochemical efficiency and bioreactor design. International Journal of Hydrogen Energy 2002;27(11):1195–208.
[88] Vasudevan P, Briggs M. Biodiesel production—current state of the art and challenges. Journal of Industrial Microbiology and Biotechnology 2008;35(5):421–30.
[89] Bolton JR, Hall DO. The maximum efficiency of photosynthesis. Photochemistry and Photobiology 1991;53(4):545–8.
[90] Doucha J, Lívanský K. Outdoor open thin-layer microalgal photobioreactor: potential productivity. Journal of Applied Phycology 2008.
[91] Doucha J, Lívanský K. Productivity, CO_2/O_2 exchange and hydraulics in outdoor open high density microalgal (*Chlorella* sp.) photobioreactors operated in a Middle and Southern European climate. Journal of Applied Phycology 2006;18(6):811–26.
[92] Hase R, Oikawa H, Sasao C, Morita M, Watanabe Y. Photosynthetic production of microalgal biomass in a raceway system under greenhouse conditions in Sendai city. Journal of Bioscience and Bioengineering 2000;89(2): 157–63.
[93] Xia J, Gao K. Effects of doubled atmospheric CO_2 concentration on the photosynthesis and growth of *Chlorella pyrenoidosa* cultured at varied levels of light. Fisheries Science 2003;69(4):767–71.
[94] Morita M, Watanabe Y, Saiki H. Photosynthetic productivity of conical helical tubular photobioreactor incorporating *Chlorella sorokiniana* under field conditions. Biotechnology and Bioengineering 2002;77(2):155–62.
[95] Pirt SJ, Lee YK, Richmond A, Pirt MW. The photosynthetic efficiency of *Chlorella* biomass growth with reference to solar energy utilization. Journal of Chemical Technology & Biotechnology 1980;30:25–34.
[96] Hall DO, Acién Fernández FG, Cañizares Guerrero E, Krishna Rao K, Molina Grima E. Outdoor helical tubular photobioreactors for microalgal production: modeling of fluid-dynamics and mass transfer and assessment of biomass productivity. Biotechnology and Bioengineering 2003;82(1):62–73.
[97] Acién Fernández FG, García Camacho F, Sánchez Pérez JA, Fernández Sevilla JM, Molina Grima E. Modeling of biomass productivity in tubular photobioreactors for microalgal cultures: effects of dilution rate, tube diameter, and solar irradiance. Biotechnology and Bioengineering 1998;58(6):605–16.
[98] Tamiya H. Mass culture of algae. Annual Review of Plant Physiology 1957;8:309–33.
[99] Laws EA, Taguchi S, Hirata J, Pang L. High algal production rates achieved in a shallow outdoor flume. Biotechnology and Bioengineering 1986;28:191–7.
[100] Minowa T, Yokoyama S-y, Kishimoto M, Okakura T. Oil production from algal cells of *Dunaliella tertiolecta* by direct thermochemical liquefaction. Fuel 1995;74(12):1735–8.
[101] Rosenberg JN, Oyler GA, Wilkinson L, Betenbaugh MJ. A green light for engineered algae redirecting metabolism to fuel a biotechnology revolution. Current Opinion in Biotechnology 2008;19(5):430–6.

[102] Sheehan J, Dunahay T, Benemann JR, Roessler P. A look back a the U.S. Department of Energy's Aquatic Species Program—biodiesel from algae. U.S. Department of Energy; 1998.

[103] Bruton T, Lyons H, Lerat Y, Stanley M, Rasmussen MB. A review of the potential of marine algae as a source of biofuel in Ireland. Dublin: Sustainable Energy Ireland; 2009. p. 88.

[104] de Morais MG, Costa JAV. Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. Energy Conversion and Management 2007;48(7):2169–73.

[105] Yoo C, Jun S-Y, Lee J-Y, Ahn C-Y, Oh H-M. Selection of microalgae for lipid production under high levels carbon dioxide. Bioresource Technology 2010;101(1, Supplement 1):S71–4.

[106] Dunahay T, Jarvis E, Dais S, Roessler P. Manipulation of microalgal lipid production using genetic engineering. Applied Biochemistry and Biotechnology 1996;57–58(1):223–31.

[107] León-Bañares R, González-Ballester D, Galván A, Fernández E. Transgenic microalgae as green cell-factories. Trends in Biotechnology 2004;22(1):45–52.

[108] Hu Q, Sommerfeld M, Jarvis E, Ghirardi ML, Posewitz MC, Seibert M, et al. Microalgal triacyglycerols as feedstocks for biofuel production. The Plant Journal 2008;54:621–39.

[109] Widjaja A, Chien C-C, Ju Y-H. Study of increasing lipid production from fresh water microalgae *Chlorella vulgaris*. Journal of the Taiwan Institute of Chemical Engineers 2009;40(1):13–20.

[110] Weldy CS, Huesemann M. Lipid production by *Dunaliella salina* in batch culture: effects of nitrogen limitation and light intensity. US Department of Energy Journal of Undergraduate Research 2007;7(1):115–22.

[111] Roessler PG. Environmental control of glycerolipid metabolism in microalgae: commercial implications and future research directions. Journal of Phycology 1990;26:393–9.

[112] Wu W-T, Hsieh C-H. Cultivation of microalgae for optimal oil production. Journal of Biotechnology 2008;136(Suppl. 1):S521–1521.

[113] Benemann JR, Weissman J, Koopman BL, Oswald WJ. Energy production with microalgae. Nature 1977;268:19–23.

[114] Weissman J, Raymond PG, Benemann JR. Mixing, carbon utilization and oxygen accumulation. Biotechnology and Bioengineering 1988;31:336–44.

[115] Weissman J, Goebel RP, Benemann JR. Photobioreactor design: comparison of open ponds and tubular reactors. Biotechnology and Bioengineering 1988;31:336–44.

[116] Brown LM, Zeiler KG. Aquatic biomass and carbon dioxide trapping. Energy Conversion and Management 1993;34(9–11):1005–13.

[117] Kadam KL. Power plant flue gas as a source of CO_2 for microalgae cultivation: economic impact of different process options. Energy Conversion and Management 1997;38(Suppl. 1):S505–10.

[118] Chelf P, Brown LM, Wyman CE. Aquatic biomass resources and carbon dioxide trapping. Biomass and Bioenergy 1991;4:175–83.

[119] Meng X, Yang J, Xu X, Zhang L, Nie Q, Xian M. Biodiesel production from oleaginous microorganisms. Renewable Energy 2009;34(1):1–5.

[120] Reith JH, van Zessen E, van der Drift A, den Uil H, Snelder E, Balke J, et al. Microalgal mass cultures for co-production of fine chemicals and biofuels and water purification. In: CODON symposium on marine biotechnology: an ocean full of prospects?; 2004. p. 16..

[121] Benemann JR, Van Olst JC, Massingill MJ, Weissman JC, Brune DE. The controlled eutrophication process: using microalgae for CO_2 utilization and agricultural fertilizer recycling. In: Gale J, Kaya Y, editors. Greenhouse gas control technologies—6th international conference. Oxford: Pergamon; 2003. p. 1433–8.

[122] Stepan DJ, Shockey RE, Moe TA, Dorn R. Carbon dioxide sequestering using microalgae systems. Pittsburgh, PA: U.S. Department of Energy; 2002.

[123] Yun Y-S, Lee SB, Park JM, Lee C-I, Yang J-W. Carbon dioxide fixation by algal cultivation using wastewater nutrients. Journal of Chemical Technology & Biotechnology 1997;69(4):451–5.

[124] de Morais MG, Costa JAV. Biofixation of carbon dioxide by *Spirulina* sp. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. Journal of Biotechnology 2007;129(3):439–45.

[125] Chang EH, Yang SS. Some characteristics of microalgae isolated in Taiwan for biofixation of carbon dioxide. Botanical Bulletin of Academia Sinica 2003;44(1):43–52.

[126] van Harmelen T, Oonk H. Microalgae biofixation processes: applications and potential contributions to greenhouse gas mitigation options. Apeldoom, The Netherlands: International Network on Biofixation of CO_2 and Greenhouse Gas Abatement with Microalgae; 2006.

[127] Benemann JR. CO_2 mitigation with microalgae systems. Energy Conversion and Management 1997;38(Suppl. 1):475–9.

[128] Muñoz R, Guieysse B. Algal-bacterial processes for the treatment of hazardous contaminants: a review. Water Research 2006;40(15):2799–815.

[129] Li Y, Horsman M, Wu N, Lan C, Dubois-Calero N. Biofuels from microalgae. Biotechnology Progress 2008;24(4):815–20.

[130] Lundquist TJ. Production of algae in conjunction with wastewater treatment. In: NREL—AFOSR workshop on algal oil for jet fuel production; 2008.

[131] Sawayama S, Inoue S, Dote Y, Yokoyama S-Y. CO_2 fixation and oil production through microalgae. Energy Conversion and Management 1995;36(6–9):729–31.

[132] Martínez ME, Sánchez S, Jiménez JM, El Yousfi F, Muñoz L. Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*. Bioresource Technology 2000;73(3):263–72.

[133] Gomez Villa H, Voltolina D, Nieves M, Pina P. Biomass production and nutrient budget in outdoor cultures of *Scenedesmus obliquus* (chlorophyceae) in artificial wastewater, under the winter and summer conditions of Mazatlán, Sinaloa, Mexico. Vie et milieu 2005;55(2):121–6.

[134] Hodaifa G, Martinez ME, Sanchez S. Use of industrial wastewater from olive-oil extraction for biomass production of *Scenedesmus obliquus*. Bioresource Technology 2008;99(5):1111–7.

[135] Muñoz R, Köllner C, Guieysse B. Biofilm photobioreactors for the treatment of industrial wastewaters. Journal of Hazardous Materials 2009;161(1):29–34.

[136] Chojnacka K, Chojnacki A, Górecka H. Biosorption of Cr^{3+}, Cd^{2+} and Cu^{2+} ions by blue-green algae *Spirulina* sp.: kinetics, equilibrium and the mechanism of the process. Chemosphere 2005;59(1):75–84.

[137] Gudin C, Therpenier C. Bioconversion of solar energy into organic chemicals by microalgae. Advances in Biotechnological Processes 1986;6:73–110.

[138] Benemann JR, Oswald WJ. Systems and economic analysis of microalgae ponds for conversion of CO_2 to biomass. US Department of Energy, Pittsburgh Energy Technology Centre; 1996.

[139] Olaizola M. Commercial development of microalgal biotechnology: from the test tube to the marketplace. Biomolecular Engineering 2003;20(4–6):459–66.

[140] Molina Grima E, Belarbi EH, Acién Fernández FG, Robles Medina A, Chisti Y. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnology Advances 2003;20(7–8):491–515.

[141] Knuckey RM, Brown MR, Robert R, Frampton DMF. Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. Aquacultural Engineering 2006;35(3):300–13.

[142] Divakaran R, Pillai VNS. Flocculation of algae using chitosan. Journal of Applied Phycology 2002;14(5):419–22.

[143] Bosma R, van Spronsen WA, Tramper J, Wijffels RH. Ultrasound, a new separation technique to harvest microalgae. Journal of Applied Phycology 2003;15(2):143–53.

[144] Carsten O, Martin B, Thomas S, Guido B, Helmut B, Peter S, et al. Standardized ultrasound as a new method to induce platelet aggregation: evaluation, influence of lipoproteins and of glycoprotein IIb/IIIa antagonist tirofiban. European Journal of Ultrasound Official Journal of the European Federation of Societies for Ultrasound in Medicine and Biology 2001;14(2):157–66.

[145] Nurdogan Y, Oswald WJ. Tube settling rate of high-rate pond algae. Water Science Technology 1996;33:229–41.

[146] Heasman M, Diemar J, O'Connor W, Sushames T, Foulkes L. Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs—a summary. Aquaculture Research 2000;31(8–9):637–59.

[147] Mohn FH. Experiences and strategies in the recovery of biomass in mass culture of microalgae. In: Shelef G, Soeder CJ, editors. Algal biomass. Amsterdam: Elsevier; 1980. p. 547–71.

[148] Petrusevski B, Bolier G, Van Breemen AN, Alaerts GJ. Tangential flow filtration: a method to concentrate freshwater algae. Water Research 1995;29(5):1419–24.

[149] MacKay D, Salusbury T. Choosing between centrifugation and crossflow microfiltration. Chemical Engineering Journal 1988;477:45–50.

[150] Prakash J, Pushparaj B, Carlozzi P, Torzillo G, Montaini E, Materassi R. Microalgae drying by a simple solar device. International Journal of Solar Energy 1997;18(4):303–11.

[151] Desmorieux H, Decaen N. Convective drying of spirulina in thin layer. Journal of Food Engineering 2006;66(4):497–503.

[152] Leach G, Oliveira G, Morais R. Spray-drying of *Dunaliella salina* to produce a β-carotene rich powder. Journal of Industrial Microbiology and Biotechnology 1998;20(2):82–5.

[153] Molina Grima E, Medina A, Giménez A, Sánchez Pérez J, Camacho F, García Sánchez J. Comparison between extraction of lipids and fatty acids from microalgal biomass. Journal of the American Oil Chemists' Society 1994;71(9):955–9.

[154] Nindo CI, Tang J. Refractance window dehydration technology: a novel contact drying method. Drying Technology 2007;25:37–48.

[155] Heger M. A new processing scheme for algae biofuels. Technology review, Available from: http://www.technologyreview.com/energy/22572/; 2009 [cited 21.05.09].

[156] Sialve B, Bernet N, Bernard O. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnology Advances 2009;27(4):409–16.

[157] Middelberg APJ. The release of intracellular bioproducts. In: Subramanian G, editor. Bioseparation and bioprocessing: a handbook. Wiley; 1994. p. 131–64.

[158] Mendes-Pinto MM, Raposo MFJ, Bowen J, Young AJ, Morais R. Evaluation of different cell disruption processes on encysted cells of *Haematococcus pluvialis*: effects on astaxanthin recovery and implications for bio-availability. Journal of Applied Phycology 2001;13(1):19–24.

[159] Amin Hejazi M, Wijffels RH. Milking of microalgae. Trends in Biotechnology 2004;22(4):189–94.

[160] McKendry P. Energy production from biomass (part 2): conversion technologies. Bioresource Technology 2002;83(1):47–54.

[161] McKendry P. Energy production from biomass (part 1): overview of biomass. Bioresource Technology 2002;83(1):37–46.

[162] Tsukahara K, Sawayama S. Liquid fuel production using microalgae. Journal of the Japan Petroleum Institute 2005;48(5):251–9.

[163] Clark J, Deswarte F. Introduction to chemicals from biomass. In: Stevens CV, editor. Wiley series in renewable resources. John Wiley & Sons; 2008.

[164] Demirbas A. Biomass resource facilities and biomass conversion processing for fuels and chemicals. Energy Conversion and Management 2001; 42(11):1357–78.

[165] McKendry P. Energy production from biomass (part 3): gasification technologies. Bioresource Technology 2002;83(1):55–63.

[166] Minowa T, Sawayama S. A novel microalgal system for energy production with nitrogen cycling. Fuel 1999;78(10):1213–5.

[167] Patil V, Tran K-Q, Giselrãd HR. Towards sustainable production of biofuels from microalgae. International Journal of Molecular Sciences 2008; 9(7):1188–95.

[168] Goyal HB, Seal D, Saxena RC. Bio-fuels from thermochemical conversion of renewable resources: a review. Renewable and Sustainable Energy Reviews 2008;12(2):504–17.

[169] Dote Y, Sawayama S, Inoue S, Minowa T, Yokoyama S-y. Recovery of liquid fuel from hydrocarbon-rich microalgae by thermochemical liquefaction. Fuel 1994;73(12):1855–7.

[170] Demirbas A. Oily products from mosses and algae via pyrolysis. Energy Sources Part A—Recovery Utilization and Environmental Effects 2006;28(10):933–40.

[171] Bridgwater AV. IEA bioenergy 27th update: biomass pyrolysis. Biomass and Bioenergy 2007;31:VII–XVIII.

[172] Chiaramonti D, Oasmaa A, Solantausta Y. Power generation using fast pyrolysis liquids from biomass. Renewable and Sustainable Energy Reviews 2007;11(6):1056–86.

[173] Miao X, Wu Q. High yield bio-oil production from fast pyrolysis by metabolic controlling of *Chlorella protothecoides*. Journal of Biotechnology 2004; 110(1):85–93.

[174] Miao X, Wu Q, Yang C. Fast pyrolysis of microalgae to produce renewable fuels. Journal of Analytical and Applied Pyrolysis 2004;71(2):855–63.

[175] Demirbas A. Oily products from mosses and algae via pyrolysis. Energy Sources Part A Recovery Utilization and Environmental Effects 2006; 28(10):933–40.

[176] USDOE. Roadmap for biomass technologies in the United States. U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy; 2002.

[177] EU. Biomass conversion technologies: achievements and prospects for heat and power generation. EUR 18029 EN. European Commission Directorate-General Science, Research and Development; 1999, 178.

[178] Olguín EJ. The cleaner production strategy applied to animal production. In: Olguín EJ, Sánchez G, Hernández E, editors. Environmental biotechnology and cleaner bioprocesses. London: Taylor & Francis; 2000. p. 227–43.

[179] Phang SM, Miah MS, Yeoh BG, Hashim MA. Spirulina cultivation in digested sago starch factory wastewater. Journal of Applied Phycology 2000; 12(3):395–400.

[180] Yen H-W, Brune DE. Anaerobic co-digestion of algal sludge and waste paper to produce methane. Bioresource Technology 2007;98(1):130–4.

[181] Ueno Y, Kurano N, Miyachi S. Ethanol production by dark fermentation in the marine green alga, *Chlorococcum littorale*. Journal of Fermentation and Bioengineering 1998;86(1):38–43.

[182] Greenbaum E. Energetic efficiency of hydrogen photoevolution by algal water splitting. Biophysical Journal 1988;54(2):365–8.

[183] Miura Y, Akano T, Fukatsu K, Miyasaka H, Mizoguchi T, Yagi K, et al. Hydrogen production by photosynthetic microorganisms. Energy Conversion and Management 1995;36(6–9):903–6.

[184] Fouchard S, Pruvost J, Degrenne B, Legrand J. Investigation of H_2 production using the green microalga *Chlamydomonas reinhardtii* in a fully controlled photobioreactor fitted with on-line gas analysis. International Journal of Hydrogen Energy 2008;33(13):3302–10.

[185] Melis A. Green alga hydrogen production: progress, challenges and prospects. International Journal of Hydrogen Energy 2002;27(11–12):1217–28.

[186] Melis A, Happe T. Hydrogen production. Green algae as a source of energy. Plant Physiology 2001;127(3):740–8.

[187] Demirbas A. Progress and recent trends in biodiesel fuels. Energy Conversion and Management 2009;50(1):14–34.

[188] Sharma YC, Singh B. Development of biodiesel: current scenario. Renewable and Sustainable Energy Reviews 2009;13(6–7):1646–51.

[189] Jet fuel from microalgal lipids. National Renewable Energy Laboratory; 2006

[190] Sheehan J, Camobreco V, Duffield J, Graboski M, Shapouri H. An overview of biodiesel and petroleum diesel life cycles. National Renewable Energy Laboratory (NREL) and US Department of Energy (USDOE); 1998.

[191] Norton TA, Melkoniam M, Anderson RA. Algal biodiversity. Phycologia 1996;35(308–326).

[192] Pulz O, Gross W. Valuable products from biotechnology of microalgae. Applied Microbiology and Biotechnology 2004;65(6):635–48.

[193] Yamaguchi K. Recent advances in microalgal bioscience in Japan, with special reference to utilization of biomass and metabolites: a review. Journal of Applied Phycology 1996;8(6):487–502.

[194] Cox PA, Banack SA, Murch SJ, Rasmussen U, Tien G, Bidigare RR, et al. Diverse taxa of cyanobacteria produce Î²-N-methylamino-l-alanine, a neurotoxic amino acid. Proceedings of the National Academy of Sciences of the United States of America 2005;102(14):5074–8.

[195] Brown MR, Jeffrey SW, Volkman JK, Dunstan GA. Nutritional properties of microalgae for mariculture. Aquaculture 1997;151(1–4):315–31.

[196] Muller-Feuga A. The role of microalgae in aquaculture: situation and trends. Journal of Applied Phycology 2000;12(3):527–34.

[197] Chuntapa B, Powtongsook S, Menasveta P. Water quality control using *Spirulina platensis* in shrimp culture tanks. Aquaculture 2003;220(1–4):355–66.

[198] Marris E. Putting the carbon back: black is the new green. Nature 2006;442(7103):624–6.

[199] Lal R. Black and buried carbons' impacts on soil quality and ecosystem services. Soil and Tillage Research 2008;99(1):1–3.

[200] Lehmann J, Gaunt J, Rondon M. Bio-char sequestration in terrestrial ecosystems. Mitigation and Adaptation Strategies for Global Change 2006;11:395–419.

[201] Lehmann J. A handful of carbon. Nature 2007;447(7141):143–4.

[202] Reijnders L. Are forestation, bio-char and landfilled biomass adequate offsets for the climate effects of burning fossil fuels? Energy Policy 2009; 37(8):2839–41.

[203] Hu C, Li M, Li J, Zhu Q, Liu Z. Variation of lipid and fatty acid compositions of the marine microalga *Pavlova viridis* (Prymnesiophyceae) under laboratory and outdoor culture conditions. World Journal of Microbiology and Biotechnology 2008;24(7):1209–14.

[204] Anonymous. FDA announces qualified health claims for omega-3 fatty acids, Available from: http://www.fda.gov/bbs/topics/news/2004/NEW01115.html; 2004 [cited 24.05.09].

[205] Ruxton CHS, Reed SC, Simpson MJA, Millington KJ. The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. Journal of Human Nutrition and Dietetics 2007;20(3):275–85.

[206] García-González M, Moreno J, Manzano JC, Florencio FJ, Guerrero MG. Production of *Dunaliella salina* biomass rich in 9-cis-[beta]-carotene and lutein in a closed tubular photobioreactor. Journal of Biotechnology 2005;115(1):81–90.

[207] León R, Martín M, Vigara J, Vilchez C, Vega JM. Microalgae mediated photoproduction of [beta]-carotene in aqueous–organic two phase systems. Biomolecular Engineering 2003;20(4–6):177–82.

[208] Guerin M, Huntley ME, Olaizola M. *Haematococcus astaxanthin*: applications for human health and nutrition. Trends in Biotechnology 2003;21(5):210–6.

[209] Waldenstedt L, Inborr J, Hansson I, Elwinger K. Effects of astaxanthin-rich algal meal (*Haematococcus pluvalis*) on growth performance, caecal campylobacter and clostridial counts and tissue astaxanthin concentration of broiler chickens. Animal Feed Science and Technology 2003;108(1–4):119–32.

[210] Lorenz RT, Cysewski GR. Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin. Trends in Biotechnology 2000;18(4): 160–7.

[211] Bermejo Román R, Alvárez-Pez JM, Acién Fernández FG, Molina Grima E. Recovery of pure B-phycoerythrin from the microalga Porphyridium cruentum. Journal of Biotechnology 2002;93(1):73–85.

[212] Viskari PJ, Colyer CL. Rapid extraction of phycobiliproteins from cultured cyanobacteria samples. Analytical Biochemistry 2003;319(2):263–71.

[213] Hirata T, Tanaka M, Ooike M, Tsunomura T, Sakaguchi M. Antioxidant activities of phycocyanobilin prepared from *Spirulina platensis*. Journal of Applied Phycology 2000;12(3):435–9.

[214] Richmond A, Lichtenberg E, Stahl B, Vonshak A. Quantitative assessment of the major limitations on productivity of *Spirulina platensis* in open raceways. Journal of Applied Phycology 1990;2(3):195–206.

[215] Morais MG, Radmann EM, Andrade MR, Teixeira GG, Brusch LRF, Costa JAV. Pilot scale semicontinuous production of Spirulina biomass in southern Brazil. Aquaculture 2009;294(1–2):60–4.

[216] Pushparaj B, Pelosi E, Tredici M, Pinzani E, Materassi R. As integrated culture system for outdoor production of microalgae and cyanobacteria. Journal of Applied Phycology 1997;9(2):113–9.

[217] Camacho Rubio F, Acién Fernández FG, Sánchez Pérez JA, García Camacho F, Molina Grima E. Prediction of dissolved oxygen and carbon dioxide concentration profiles in tubular photobioreactors for microalgal culture. Biotechnology and Bioengineering 1999;62(1):71–86.

[218] Acién Fernández FG, Fernandez Sevilla JM, Sanchez Perez JA, Molina Grima E, Chisti Y. Airlift-driven external-loop tubular photobioreactors for outdoor production of microalgae: assessment of design and performance. Chemical Engineering Science 2001;56(8):2721–32.

[219] Carlozzi P. Dilution of solar radiation through "culture" lamination in photobioreactor rows facing south-north: a way to improve the efficiency of light utilization by cyanobacteria (*Arthrospira platensis*). Biotechnology and Bioengineering 2003;81(3):305–15.

[220] Garcia-Malea Lopez MC, Del Rio Sanchez E, Casas Lopez JL, Acién Fernández FG, Fernandez Sevilla JM, Rivas J, et al. Comparative analysis of the outdoor culture of *Haematococcus pluvialis* in tubular and bubble column photobioreactors. Journal of Biotechnology 2006;123(3):329–42.

[221] Cheng-Wu Z, Zmora O, Kopel R, Richmond A. An industrial-size flat plate glass reactor for mass production of *Nannochloropsis* sp. (Eustigmatophyceae). Aquaculture 2001;195(1–2):35–49.

[222] Converti A, Lodi A, Del Borghi A, Solisio C. Cultivation of *Spirulina platensis* in a combined airlift-tubular reactor system. Biochemical Engineering Journal 2006;32(1):13–8.

[223] Carlozzi P. Hydrodynamic aspects and Arthrospira growth in two outdoor tubular undulating row photobioreactors. Applied Microbiology and Biotechnology 2000;54(1):14–22.

[224] Chini Zittelli G, Rodolfi L, Biondi N, Tredici MR. Productivity and photosynthetic efficiency of outdoor cultures of *Tetraselmis suecica* in annular columns. Aquaculture 2006;261(3):932–43.

[225] Sato T, Usui S, Tsuchiya Y, Kondo Y. Invention of outdoor closed type photobioreactor for microalgae. Energy Conversion and Management 2006;47(6):791–9.
[226] Graverholt O, Eriksen N. Heterotrophic high-cell-density fed-batch and continuous-flow cultures of *Galdieria sulphuraria* and production of phycocyanin. Applied Microbiology and Biotechnology 2007;77(1):69–75.
[227] Xiong W, Li X, Xiang J, Wu Q. High-density fermentation of microalga *Chlorella protothecoides* in bioreactor for microbio-diesel production. Applied Microbiology and Biotechnology 2008;78(1):29–36.
[228] Wu Z, Shi X. Optimization for high-density cultivation of heterotrophic *Chlorella* based on a hybrid neural network model. Letters in Applied Microbiology 2007;44(1):13–8.
[229] de Swaaf ME, Sijtsma L, Pronk JC. High-cell-density fed-batch cultivation of the docosahexaenoic acid producing marine alga *Crypthecodinium cohnii*. Biotechnology and Bioengineering 2003;81(6):666–72.
[230] de Swaaf ME, Pronk JT, Sijtsma L. Fed-batch cultivation of the docosahexaenoic-acid-producing marine alga *Crypthecodinium cohnii* on ethanol. Applied Microbiology and Biotechnology 2003;61(1):40–3.
[231] Chiu S-Y, Kao C-Y, Chen C-H, Kuan T-C, Ong S-C, Lin C-S. Reduction of CO_2 by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor. Bioresource Technology 2008;99(9):3389–96.
[232] Scragg AH, Illman AM, Carden A, Shales SW. Growth of microalgae with increased calorific values in a tubular bioreactor. Biomass and Bioenergy 2002;23(1):67–73.
[233] Fukuda H, Kondo A, Noda H. Biodiesel fuel production by transesterification of oils. Journal of Bioscience and Bioengineering 2001;92(5):405–16.
[234] Song D, Fu J, Shi D. Exploitation of oil-bearing microalgae for biodiesel. Chinese Journal of Biotechnology 2008;24(3):341–8.
[235] Xu H, Miao X, Wu Q. High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. Journal of Biotechnology 2006;126(4):499–507.
[236] Ratledge C. Fatty acid biosynthesis in microorganisms being used for single cell oil production. Biochimie 2004;86(11):807–15.

Liam Brennan is a PhD student in the Charles Parsons Energy Research Programme of Science Foundation Ireland, University College Dublin. His research area of interest is in algal biomass-to-biofuels covering identification of algal species for oil extraction, optimisation of production systems, conversion technologies and environmental impact assessment.

Dr. Philip Owende is a senior research fellow in the Charles Parsons Energy Research Programme of Science Foundation Ireland, University College Dublin. His research area of interest is in biomass-to-energy systems covering energy feedstock production, pretreatment standards, conversion technologies, and environmental impact assessment. He has published 35 scientific articles in peer reviewed journals, over 30 international conference papers, and three book chapters.